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FEEDING AND DIGESTION IN
Phoronis vancouverensis PIXELL;
A MORPHOLOGICAL, ULTRA-STRUCTURAL
AND ENZYMATIC STUDY

by

©

JOHN HENRI VANDERMEULEN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Feeding and Digestion in Phoronis vancouverensis Pixell; a morphological, ultra-structural and enzymatic study", submitted by John Henri Vandermeulen in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Feeding and digestion in the Phoronida is re-examined in *Phoronis vancouverensis* Pixell with histochemical, electrophoretic, and electron microscopic developments. Particulate matter is drawn into the lophophore by metachronal beating of lateral cilia on the tentacles. Oral cilia do not play a role in feeding, instead acting as an ejection mechanism for rejected particles. Selection of food particles appears to be by particle size only.

Two gland cell types are distinguishable morphologically and histochemically. An acidophil gland cell, found in the lophophore and trunk epidermis, secretes a muco- or glyco-protein of unknown function. A basiphil gland cell, found in the trunk epidermis, buccal groove and digestive tract, produces a mucoid secretion, probably a chondroitin sulfate.

The digestive tract consists of four functionally different regions. Evidence suggests the oesophagus is the region of ingestion and conduction by epithelial ciliation of food particles. The proventriculus and stomach are typified by dense ciliation, presence of zymogen granules, and strong esterase and phosphatase activity. The proventriculus is suggested as the site of lipid metabolism. The stomach appears to be the site of extra-cellular digestion. The intestinal epithelium lined with numerous microvilli and with absorptive vesicles in the supra-nuclear cytoplasma is the site of distal absorption and intra-cellular digestion.

A study of hydrolytic enzymes reveals the presence of seven esterases, three alkaline phosphatases, one acid phosphatase, four gelatinases, and one amylase. Preliminary investigations indicate several of these enzymes operate at different pH values, and are subject to different activation mechanisms.

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I. GENERAL INTRODUCTION

The phylum Phoronida consists of two genera of little known oligomerous vermiform eucoelomates. First scientific notice of the phylum is that of Müller (1846), with a description of the actinotroch larva. During the period from its first mention until the turn of the century a spate of literature on these organisms was to be found in the various zoological and biological journals. Noteworthy is the monograph on the adult form by Selys-Longchamps (1907). Phoronis vancouverensis received its first mention in 1912 by Pixell. Following the early interest in this group there is little literature until such latter day works as those by Cori (1939), Lönöy (1954), Silèn (1952, 1954a,b, 1955, 1956), Rattenbury (1953, 1954), Marsden (1957), Robin (1964) and Zimmer (1967).

Most of the work on this organism has been taxonomic, embryological, or dealing with regeneration. Taxonomic, since early in its description the Phoronida appeared to be a possible link between the lower invertebrates and the hemichordates (Gilchrist, 1907). Also, since the organism is found world-wide, it lends itself to distribution and comparative physiological studies. Embryological, since the larvae occur in large numbers, in some species are brooded within the lophophoral cavity and are thus readily available, and are reared easily in a well-equipped laboratory. Regeneration, since the organism is capable of shedding the lophophore, each resulting part of the organism in turn capable of regenerating a complete individual (Cori, 1890; Schultz, 1905; Harmer, 1917; Gilchrist, 1919; Silèn, 1955; Marsden, 1957).

Physiological studies, and observations on feeding mechanism and digestion are limited. The first, and it appears the only, description of the feeding behavior in this group seems to be that of Gilchrist (1907), based on *Ph. capensis*. Pixell (1912), in her description of *Ph. vancouverensis*, suggests that digestion occurs intracellularly, but gives no further observations. Little is known about the presence of digestive regions, nutrient transport in the gut, digestion rates, or enzymes. Becker (1939), in studying digestion in the actinotroch larva, assumed that the same results would apply to the adult phoronid. Ohuye (1942) in a paper on haematopoiesis in *Ph. australis* Haswell and *Ph. ijimai* Oka, describes the passage of carmine grains through the gut epithelium and the surrounding basement membrane into the trunk coelom. Marcus (1949) describes diatom skeletons within gut epithelium cells in *Ph. ovalis* Wright, suggesting holophagic, intracellular digestion. Dawydoff *et al.* (1959) describe a partial syncytium in the stomach epithelium of *Ph. ovalis*, "apparently capable of phagocytosis of particulate matter in the gut". They concede, however, the possibility of extracellular digestion, especially in view of the apparent enzymatic nature of the gastric fluids.

It was in view of the foregoing that a preliminary study of the feeding mechanism of the Phoronida was begun in the summer of 1965 at the University of Washington's Friday Harbor Laboratories. The initial observations were followed up with a more intensive study of the digestive processes. Emphasis was placed on correlating histological and histochemical findings with *in vivo* observations in order to arrive at some conclusions regarding the functioning of the gut. In addition, an attempt was made to determine the extent of intracellular and extracellular digestion in this group. To

these ends, both light and electron microscopy, as well as histochemical techniques for assessing enzyme activity, and electrophoretic characterization of some of the hydrolytic enzymes were employed.

Phase microscopy and electron microscopy were deemed desirable in this study, since most of the histological descriptions about this phylum date back to the 19th century and the first decade of the 20th century. Indeed, many of the illustrations on this group in "The Invertebrates" (Hyman, 1959) are based on drawings by Selys-Longchamps (1907).

Because of the two-fold approach of this study, the thesis has been sub-divided into two parts. The first part consists of a description of the structure of the lophophore as a feeding mechanism and a description of this activity, as well as a brief morphological description and a histochemical analysis of certain gland cells in the tentacle epidermis. The second part concerns the study of the digestive tract, its histology as revealed primarily by electron microscopy, and a correlation of these findings with a histochemical and electrophoretic survey of certain digestive enzymes.

II. MATERIALS AND METHODS

A. Collection

The specimens of Ph. vancouverensis Pixell used in this study were collected at San Juan Island, Washington, and Departure Bay, Vancouver Island.

The San Juan Island specimens were taken from the silt covering the rocky substrate in Westcott Bay, and from a similar substrate in Garrison Bay. Both of these locations lie intertidally, in quiet waters, well inland from the exposed outer seashores of the island.

The Departure Bay specimens were collected from a group of small rocky (cretaceous sandstone) islands in the center of Departure Bay, almost directly opposite the Fisheries Research Board of Canada Biological Station. The specimens were found on the underside of crevices and concave rock surfaces, and were exposed to the action of waves and weather, being covered by water at moderately high tides, and exposed to wave action and air at low tides.

Specimens were transported back to the laboratories in ice-chests and maintained in circulating seawater tanks. Those collected on San Juan Island and studied at the Friday Harbor Laboratories were kept in circulating seawater tanks. Those obtained at Departure Bay were brought to the University of Victoria, maintained and studied in seawater hauled daily from Oak Bay. Only specimens freshly taken from the sea were used in the following studies.

B. Gross Observations

Clusters of phoronids (circa nine square centimeters) (FRONTISPIECE) were examined in individual six inch fingerbowls which could easily be taken from the aerated circulating seawater tanks for observation. Gross observations were made with binocular dissecting microscopes, whereas individual tentacles and ciliary action were studied by examining excised portions of the lophophore with a Wild M20 microscope equipped for phase contrast.

Movement of small particles in the seawater usually rendered visible water current patterns and general feeding behavior of Ph. vancouverensis. Particular areas of interest were more closely examined by sprinkling carmine powder and sand grains on the surface of the water directly over the lophophore, or by introducing these particles directly to the portion of the lophophore under observation.

C. Histology - Light Microscopy

Specimens marked for preservation and subsequent treatment for light microscopy were first relaxed by spreading a 1 : 1 mixture of menthol and chloral hydrate in the form of an aqueous, eutectic paste over the surface of the water, and left for approximately fifteen minutes, or until the tentacles showed no response to probing. A variety of fixatives were used for preservation (TABLE I), although no one fixative appeared to excell in preserving fine detail. Selected tissues were embedded in paraffin and 8 to 10 μ serial sections were made in both the transverse and in the longitudinal planes. Sections were stained in Heidenhain's haematoxylin with eosin counterstain or in Masson's trichrome for histological detail (Pantin, 1964). However, a variety of staining techniques was used for histological and histochemical studies of the epidermal gland cells.

D. Histology - Electron Microscopy

For electron microscopy, 36 specimens of Ph. vancouverensis were fixed in their entirety in 2% osmium tetroxide buffered with S-collidine (2,4,6-trimethyl pyridine) at pH 7.5 (Luft, 1961) for one hour at 0°C, washed in distilled water, and dehydrated in a graded series of ethanols as follows: 30%, 50%, 70%, and 95% for 10 minutes each, and two 5 minute changes in 100% ethanol. Following two changes of 10 minutes each in propylene oxide, the specimens were passed into the following mixture:

7 parts resin A (Epon 812 - DDSA)
3 parts resin A (Epon 812 - MNA)
0.16 parts DMP 30 accelerator
10 parts propylene oxide

Specimens were allowed to stand in the above mixture overnight in an uncovered vial to permit ready evaporation of the propylene oxide. Specimens were embedded in a fresh mixture of Epon containing no propylene oxide, and polymerized for 20 to 24 hours at 60°C in one centimeter square polythene ice-cube trays.

Of the 36 specimens embedded in Epon, 23 were subsequently used for thick and thin sections. Most sections were cut with glass knives on a Porter-Blum (MT-I) microtome at thicknesses varying from 600 Å (silver-gray) to 1000 Å (gold). A diamond knife (Du Pont) was used to section the lophophore and tentacles, since differences in hardness of the tissue and the surrounding Epon often made sectioning with glass knives difficult. One micron sections were made at 5 μ intervals for light microscopy. Thin sections were mounted on Formvar-coated 200-mesh copper grids.

Mounted thin sections were stained in 2% uranyl acetate in methanol for 15 minutes (Stempak et al., 1964), and briefly washed in a

graded series of methanol and distilled water. Counterstaining in 0.3% lead citrate in distilled water (Venable et al., 1965) was done for 3 minutes, followed by three rinses in distilled water.

Thick sections were mounted on glass slides and used for general scanning and locating areas of interest for subsequent electron microscopy. These sections were oxidized for 5 minutes in 1% periodic acid, then washed briefly in distilled water. Subsequently, the sections were stained on a hot plate (37°C) with a 1 : 1 mixture of 1% Azure 2 in distilled water and 1% methylene blue in 1% borax (Richardson et al., 1960) for about four minutes. After rinsing, the sections were mounted in 'Histoclad' (Clay Adams Inc., New York).

The Philips EM 200 at 80 KV was used principally throughout this study, although a Philips EM 100B was used on occasion. Micrographs were taken on 35 mm Kodak Fine Grain Positive film (P426), and developed in Kodak developer D19.

E. Enzyme and Lipid Histochemistry

For enzyme histochemistry of the various regions of the digestive tract whole living specimens were cut into short lengths, embedded in OCT compound (Can-Lab) and frozen at minus 30°C. 15 to 18 μ serial sections were cut and mounted on chilled glass slides in a Model CTD, International Harris Cryostat, and allowed to thaw and dry at room temperature. Frozen sections mounted on glass slides could be kept stored up to a week at minus 10°C, with no apparent loss of enzymatic activity.

All of the staining techniques used depended on the coupling of a substrate hydrolysis product with an appropriate diazonium salt (Sigma Chem.

Co., St. Louis). For demonstration of esterases, sections were incubated for five minutes in a solution of 1% α-naphthyl acetate in acetone, buffered to pH 7.5 with 0.1M phosphate buffer, to which 1 mg/ml Fast Blue B was added (Gomori, cited by Barka and Anderson, 1965).

For demonstration of alkaline phosphatase the substrate used was sodium-α-naphthyl acid phosphate, dissolved in 0.1M veronal acetate buffer (pH 9.2) in a concentration of 1.5 mg/ml (Menten _et_ _al_., cited by Barka and Anderson, 1965). One mg/ml of Fast Red-TR or Fast Violet-B was added to this solution, and the sections were incubated in this medium for one hour at room temperature. Sections were washed briefly in distilled water, then washed for two minutes in 1% acetic acid, and again washed in distilled water before being mounted in glycerine jelly. For acid phosphatase demonstration, sodium-α-naphthyl acid phosphate was dissolved in 0.1M veronal acetate buffer (pH 5) in a concentration of 1 mg/ml. Fast Garnet-GBC was added in a 1 mg/ml concentration (Barka and Anderson, 1965). Following incubation for twenty minutes at 37°C the sections were washed in running water and mounted in glycerin jelly.

Sudan IV was used to demonstrate the presence of lipids in frozen sections, using the procedure recommended by Barka and Anderson (1965).

F. Starch Gel Electrophoresis

Several hundred _Ph_. _vancouverensis_ were extracted from their tubes with micro-dissecting forceps, and homogenized with distilled water in a Potter homogenizer. The resulting homogenate was centrifuged in an International High-Speed Refrigerated Centrifuge (Model HR-1) at 13,000 rpm* for 30 minutes. The supernatant was filtered in a Schleicher and Schnell

---

*g-value or rotor size not noted.

Collodion Bag ultrafilter for concentration of proteins. The concentrate obtained was distributed into small vials in 0.1 ml. aliquot parts and frozen until enzyme separation was carried out.

The method of starch gel electrophoresis used in this study (Smithies, 1955) was devised primarily for separation of serum proteins, but has been shown applicable to enzyme separations (Hunter and Markert, 1957; Uriel, 1960; Reid, 1965). A 'gel' was prepared in the standard manner (Smithies, 1955), filter paper strips on to which extract aliquots had been adsorbed were inserted into the starch gel slab, and separation of enzymes in the gel was carried out at 180 volts in a standard horizontal electrophoresis tank, using a tris-maleic acid buffer at pH 8.49. After separation acid and alkaline phosphatases, esterases, and gelatinases (proteases) were localized in the gel slices. The optimal pH level for incubation of each enzyme was determined by incubating gel slices in the appropriate staining media over a range of pH values.

For demonstration of esterases, the gel slices were incubated for five minutes in a solution of 1% α-naphthyl acetate in acetone, buffered to pH 6.5 with 0.05M tris-maleic buffer, to which 1 mg/ml Fast Violet-B was added. Simultaneous 'runs' were done on fat-free extracts, the fat having been removed from extract aliquots with butanol. The substrate for demonstration of alkaline phosphatases was sodium-α-naphthyl phosphate, dissolved in 0.05M tris-maleic buffer at a pH of 8.2. The diazonium salt used was Fast Violet-B, added in a concentration of 1 mg/ml of solution. Acid phosphatases were demonstrated with the same incubating medium, but buffered to a pH of 3.8. Demonstration of acid and alkaline phosphatases was run concurrently, since alkaline phosphatase bands will appear in gel

slices stained for acid phosphatases due to the alkaline pH of the gel.

For the demonstration of gelatinases, a thin layer of 1% agar containing 0.5% gelatin was poured over each gel slice and these double layers of starch and agar gel were incubated for 12 hours at 37°C. Following incubation the agar-gelatin overlay was treated with saturated mercuric chloride in 1N HCl. This rendered the undigested gelatin as an opaque white film, the regions of gelatinase digestion appearing as clear bands, corresponding to the bands of enzymes (Reid, 1965).

G. Estimation of Gelatinase pH Optimum

The pH optimum of gelatinase demonstrated by the electrophoretic technique outlined above was estimated by a charcoal-gelatin technique (Reid, 1965). An aliquot of extract was divided among six 3 ml. glass centrifuge tubes, containing a graded pH series of tris-maleic acid buffer, ranging from pH 3.5 to pH 8.5. Into each tube was placed a portion of a charcoal-gelatin disc (Oxoid) which lodged at the top of the tapering portion of the tube. As digestion of the gelatin in the disc proceeded, the charcoal was released and fell to the bottom of the tube. A visual record of rate of disc disintegration versus optimum pH values was kept. The technique was repeated to determine whether any enzyme activation occurred after addition of cysteine hydrochloride.

H. Amylase Spot Test for Carbohydrases

Presence of carbohydrases (amylases) and optimum pH levels were determined by the following method. A 2% boiled starch solution was mixed 1 : 1 with appropriate tris-maleic buffer (pH 5.5 to pH 8.5). After addition of a small amount of extract the solution was allowed to stand

at room temperature. Samples were taken at 1 min., 5 min., 10 min., 25 min., and 45 min., and tested for amylase activity with Lugol's iodine (5% I_2 in 10% KI).

III. THE LOPHOPHORE

A. Introduction

Our knowledge of the lophophore as a feeding mechanism is based primarily on a paper by Gilchrist (1907). He writes of tentacular ciliary currents producing an "inset current", particles being carried to the mouth on the tentacles, "evidently by the active cilia". He notes, however, that "it was surprising to find that, in many cases, after reaching the base of the tentacles the same particles were returned on the same tentacles, and travelled as rapidly in the opposite direction to the distal end of the tentacle". He ascribes this seeming reversibility of ciliary beat to two regions of cilia, beating in opposite directions, lying on opposite sides of the tentacle. Cilia on the left side of the tentacle beat upward, those on the right side downward (seen from the lophophoral chamber). The direction of particle travel is, therefore, dependent on which side of the tentacle is presented to the particle. He states, however, that this point was not conclusively established, and that it is certain that the propulsive action of the cilia is not only toward the mouth, but is largely concerned in carrying away any rejected particles.

Selys-Longchamps (1907), in his monograph Phoronis, makes no distinction between cilia, only describing their presence on the oral and lateral surfaces of the tentacles. His appears to be the first notation, however, of epidermal gland cells on the aboral surface of the tentacles. He describes their appearance and secretions, but does not correlate function

with gland cell type. He suggests that the secretion must be used for greasing the body surface, tube formation, and dissolving calcareous substratum in burrowing forms.

Pixell (1912) in her study of Phoronis vancouverensis describes three gland cell types, the first containing "very fine granules", the second "much coarser spherical granules or globules, two to three microns in diameter", and the third containing "homogeneous mucus".

Cori (1939) describes a general ciliation over the tentacles of the lophophore in Ph. ovalis and Ph. hippocrepia. Some of these cilia are reported as setting up an incoming current, which after passing through the lophophore leaves between the tentacles. Particulate matter, suspended in these water currents, are described as coming into contact with the cilia, which particles are then thrown from cilium to cilium into the direction of the mouth.

Marcus (1949) describes two epidermal gland cell types in Ph. ovalis from Brazil, the first type with acidophil, and the second type with basiphil secretion. She suggests that the chitinous cuticle is produced principally by the acidophil type of cell, while the basiphil cells perhaps furnish the substance that dissolves the calcareous substratum. She describes the acidophil mucus secreting cells as long narrow cells with fine granular content, and the basiphil cells as distended cells with coarser contents.

Silèn (1952) describes two gland cell types. The first, with a "homogeneous or alveolar content", stains a clear blue with Heidenhain's azan. It is described as secreting a film over the epidermis which film also stains a clear blue with Heidenhain's azan. The second cell-type, a

"droplet containing" cell, is very numerous on the aboral faces of the tentacles. It shows no reaction with protargol or Heidenhain's azan, but reacts very positively to azo-carmine. Silèn does not ascribe a function to this second, droplet-containing cell.

Finally, Lönöy (1954) reports finding in the outer corner of tentacle cross-sections one "comparatively great cell, probably having a secretory function". Such similar cells have been described by Selys-Longchamps (1907) and Cori (1939).

Hyman (1959) throws no further light on the character of the tentacular gland cells. With respect to the lophophore as a whole, she states that it is evident from their construction and behavior that the phoronids are ciliary-mucus feeders, all particles becoming coated with mucus.

It was in order to resolve some of this uncertainty about ciliation, ciliary beat direction, role of mucus, and the character of the lophophoral gland cells that the first part of this study was undertaken.

B. General Description

The lophophore of Ph. vancouverensis Pixell consists of a horseshoe shaped or crescentic folding of a continuous ring of tentacles, forming an outer 'ventral'* and an inner 'dorsal' tentacle ring, enclosing the mouth along the mid-line (FIG. 1). Between the arms of the horseshoe shaped lophophore and dorsal to the inner tentacle ring lie the anus, nephridial openings, and in mature specimens the lophophoral organs.

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*The use of the terms 'dorsal' and 'ventral' is that adopted by Hyman (1959).

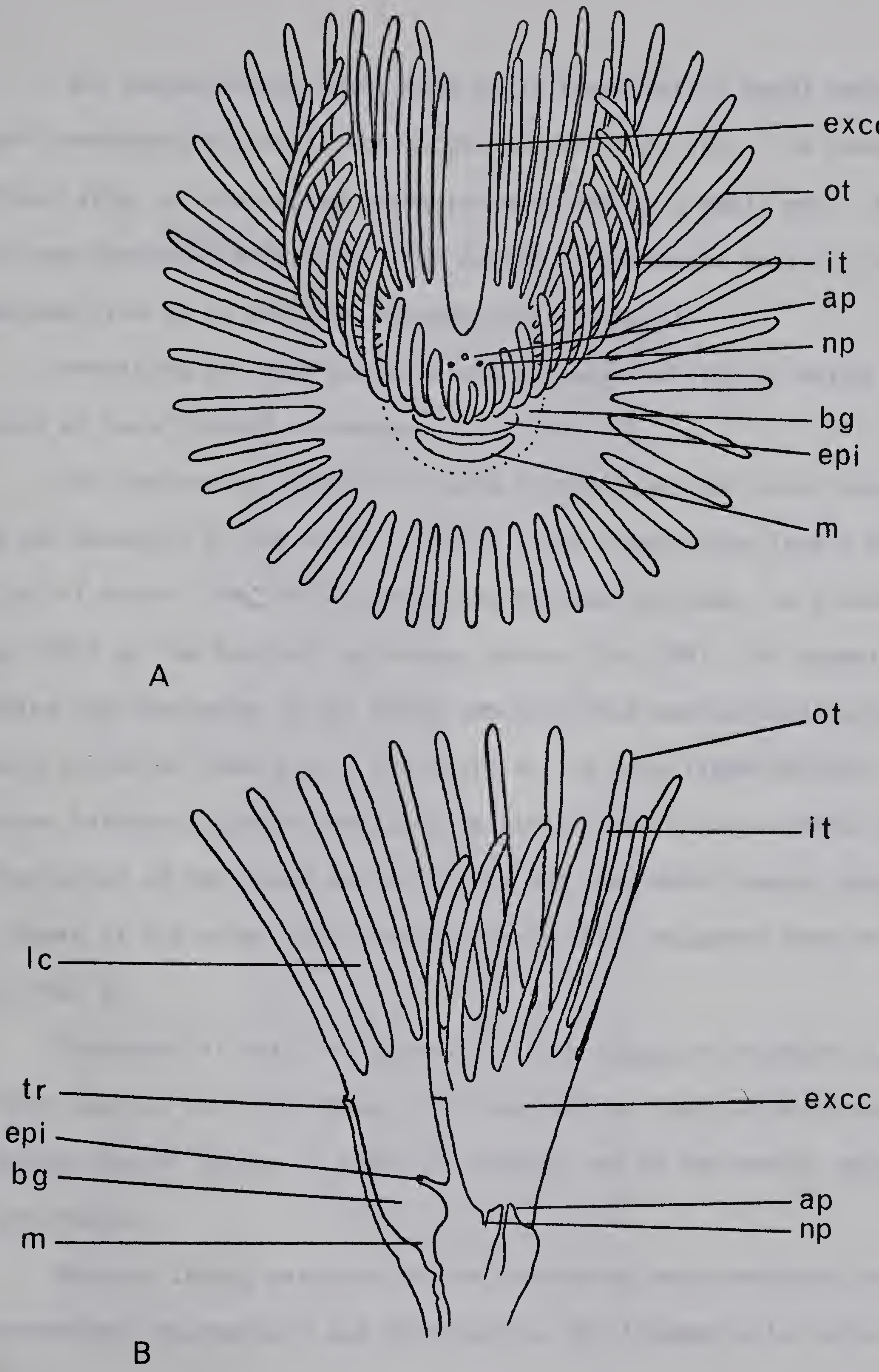

FIGURE 1. Diagrammatic representation of the lophophore of _Phoronis vancouverensis_ Pixell. A, oral view, showing lophophore chamber (after Brooks & Cowles, 1905). B, cut-away side view, showing relationship of inner and outer tentacle rings to lophophore chamber and excurrent chamber.
ap, anal papilla. bg, buccal groove. epi, epistome. excc, excurrent chamber. it, inner tentacle ring. lc, lophophore chamber. m, mouth. np, nephridiopore. ot, outer tentacle ring. tr, tentacle ridge. X 60
]

The tentacles are fused near their bases into a basal tentacle ridge*, enclosing within it the buccal groove (FIG. 1B). The inner, dorsal tentacle ring is interrupted along the mid-line by a small gap. At this point new tentacles originate. The number of tentacles varies in mature organisms from 80 to 100, the average being about 92.

Overlying the oval mouth is the epistome, a flap of solid tissue, capable of very limited movements.

The lophophore chamber, bounded ventrally by the outer tentacle ring and dorsally by the inner tentacle ring, tapers down from a width at the top of about 2 mm. when in extended feeding attitude, to a width of about 300 µ at the base of the buccal groove (FIG. 1B). In normal feeding position the tentacles of the outer tentacle ring are splayed outwards forming a slotted funnel with the mouth at its base (FRONTISPIECE). The distance between adjacent tentacles at their tips is circa 200 to 300 µ. The tentacles of the inner tentacle ring are held more closely together than those of the outer ring; approximately 100 µ separate them at their tips (FIG. 1).

Tentacles of both the outer and inner rings are heavily ciliated on their lateral and oral faces, very sparsely on their aboral sides (FIG. 2). The entire buccal groove is heavily ciliated, as is the mouth, epistome, and oesophagus.

Excised living sections of the lophophore were observed with phase-contrast microscopy, and three groups of ciliated cells were noted:

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*Tentacles and ridges will here be considered to constitute the lophophore.

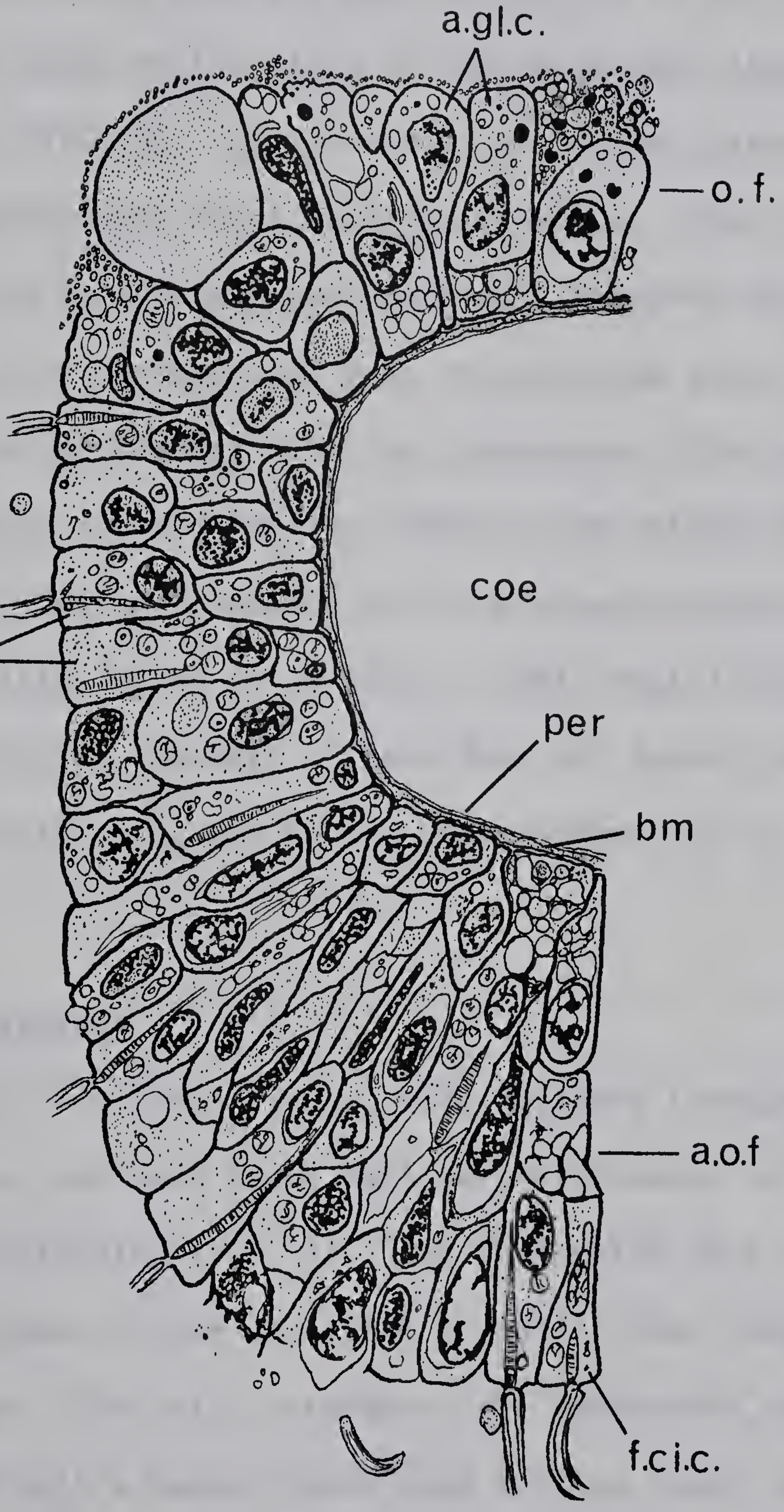


FIGURE 2. Diagrammatic representation of tentacle epidermis of Phoronis vancouverensis Pixell. a.gl.c., acidophil gland cell. a.o.f., aboral face. bm, basement membrane. coe, coelom. f.ci.c., frontal ciliated cell. l.ci.c., lateral ciliated cell. o.f., oral face. per, peritoneum.

frontal ciliated cells, lateral, and latero-frontal. The cilia of the frontal cells (FIG. 2), are shortest, circa 8 to 12 μ in length, and densely cover the entire oral faces of the tentacles. They beat very rapidly, moving particles from the base to the tip of the tentacle. The lateral cilia are found on the sides of the tentacles, lining the intertentacular spaces (FIG. 2). The laterals are longer, circa 10 to 15 μ in length, and appear more dense than the frontals. They beat in an oral to aboral direction in a metachronal fashion. Wherever the direction of ciliary beat could be observed, no beat reversal was seen, regardless whether a tentacle was severed from the lophophore. The latero-frontal cilia (FIG. 2) occur in much smaller numbers than either the frontal or lateral cilia. They are visible with the phase-contrast microscope as long individual cilia (circa 15 to 20 μ). Their beat is a single power stroke with a whip-like recovery. There does not appear to be coordination between cilia of this type, nor is the beat rhythmic or in a constant direction.

C. Lophophore Epidermis

The tentacle epidermal cells, when followed throughout their length in serial sections, are seen to be tall narrow columnar cells forming a simple columnar epithelium (FIG. 2). The epithelium over the oral and lateral, ciliated faces of the tentacles is taller than that of the aboral, non-ciliated faces. The cells throughout the lophophore epidermis are narrowed basally, with the cell's basal plasmalemma resting flatly on the underlying basement membrane, and not "on three to six diverging tips" as suggested

by Silèn (1951). The epithelium of the non-ciliated aboral tentacle face consists primarily of secretory gland cells.

Examination of thick sections shows three types of nuclei, distinguishable by their varying reactivity to stain. Electron micrographs confirm the presence of three nuclear types, distinguishable by varying density of chromatin masses (PLATE 1). It was not possible to correlate cell function, i.e. ciliated or secretory, with nuclear type, all types occurring throughout the epidermis of the lophophore. The possibility exists that the three nuclear variations are in fact different cyclical stages of a basic nuclear type. The nuclei are generally spherical or ovoid in shape, about 3 μ wide and 5 μ long, their long axis corresponding to the long axis of the cell (FIG. 2). In the epithelium of the oral faces an extremely elongated (2.5 μ wide by 6 μ long), almost pycnotic nucleus occurs frequently (FIG. 2). Whether this type is yet another cyclical variation of the basic nuclear type, or whether it is associated with a specific function is not known.

The secretory cells in the aboral epithelium of the tentacles differ from the non-secretory cells found elsewhere in the lophophore in the large number of globules scattered throughout their cytoplasm, generally in their lack of ciliation, and in the dilated appearance of the nuclear envelope (PLATE 1, 2). Globules of varying sizes are commonly found basally in these cells (FIG. 2). Rough endoplasmic reticulum is found scattered throughout the cytoplasm (PLATES 2, 3). Golgi complexes were observed rarely (PLATE 3). Mitochondria are generally spherical in appearance, some with tubular cristae (PLATE 1).

Apically, the cell membrane of some cells in the secretory aboral tentacle epithelium is thrown into numerous projections (PLATES 4, 5). Some of these are in the form of outfoldings of the distal plasma membrane (PLATE 4); others are in the form of microvilli, projecting out from the plasma membrane (PLATES 5, 6). The microvilli are long and thin, about 450 Å wide by 3600 Å long, and are enveloped in a very pronounced electron-dense coat material, consisting of a conspicuous layer of fine filaments radiating from the membrane delimiting the microvilli.

Spherules of varying sizes are found in the space surrounding the tentacle epidermis, primarily over the aboral faces (PLATE 7). These spherules can be separated into two types on the basis of coat material (PLATE 8). The first type, lacking coat material, is spherical or elongate in shape and is up to 2 μ in diameter (g, PLATES 7, 8). In appearance these resemble similar spherules found in the cytoplasm of the aboral epithelial secretory cells. The second spherule type is generally spherical, up to 0.4 μ in diameter, and is enveloped in a conspicuous layer of coat material (PLATES 8, 9, 10). More irregularly shaped structures occur in some sections (PLATE 9). Under higher magnifications a double limiting membrane is discernible in this second spherule type (PLATE 10).

Examination of tentacle and trunk epidermis of Ph. vancouverensis in paraffin sections reveals the presence of two types of gland cells. The first occurs very abundantly on the aboral faces of the tentacles and throughout the trunk epidermis. Its contents consist of numerous small (circa 0.5 μ in diameter) acidophil droplets. In paraffin sections this cell

type frequently assumes a very dilated shape. Electron microscope examination reveals many small globules scattered throughout the cytoplasm of this gland cell type; however, not in as great numbers or size as expected from light microscope observations on paraffin embedded tissue.

The second type of gland cell is not found in the tentacles, but occurs in very large numbers in the buccal groove epithelium and the trunk epidermis. This second gland cell type is characterized by its size, up to 9 µ wide, and its large (up to 2 µ in diameter) basiphil globular contents (PLATE 11). Electron micrographs confirm these light microscope findings. The contents of the cell are seen to consist of a compacted mass of large globules, increasing in size apically (PLATE 12).

Sections of ciliated cells from the different ciliated regions show the characteristic nine double peripheral and two single central fibrillar configuration. Proximally the cilium fibrils appear to end in a basal plate, which is separated from the upper end of the basal body by an indistinct zone (PLATE 13). In cross-section the cilium is circular, about 0.4 µ in diameter. Near its base, the whole cilium narrows to about 0.3 µ in diameter; proximal to this constriction the outer membrane flares out to blend continuously into the distal plasma membrane of the cell. The basal bodies appear as short, hollow, cylindrical bodies, closed at the distal end, nearest the basal plate, and terminating in the ciliary rootlet. A second basal body has been found lying at right angles to the main basal body, apparently identical in structure, lying somewhat below it. It appears attached to the rootlet by a short fibril. The ciliary rootlet originates from the main basal body at an angle of about 30°. A second, much shorter, rootlet has been found originating from this same point at about 60° (PLATE 14). The main

rootlet appears to terminate in the vicinity of the nucleus. It consists of a cross-striated fiber, approximately 200 μ in diameter, with a major cross-banding periodicity of 650 Å.

D. Gland Cell Histochemistry

Histochemically the two epidermal gland cell types are readily distinguished by their differential reactivity to a combined Alcian Blue-periodic acid Schiff (PAS) stain. The tentacular secretory cells react very positively to the PAS reaction, staining a vigorous red throughout the contents. The second gland cell type found in the buccal groove and trunk epidermis is negative to the PAS technique, but stains a deep blue with the Alcian blue (PLATE 11). The blue stain appears confined to the periphery of the inclusion bodies, or to the spaces between the globular contents. Whether this reflects uneven fixation, or uneven stain penetration, or compartmentalization of stainable materials is not known.

The strongly positive PAS reaction by the acidophil cell type indicates the presence of certain carbohydrates or conjugated carbohydrates (Pearse, 1959), while the negative reaction to the Alcian Blue indicates a lack of acid mucopolysaccharides. No change in PAS response was noted following diastase digestion, indicating absence of glycogen. No change was noted after acetylation, even after 24 hours, which indicates a lack of substances of presumed lipid character (Barka and Anderson, 1965). Sudan IV staining for fats gave a negative reaction for lipid in these gland cells, thus confirming the acetylation findings. Ninhydrin staining (Pearse, 1959) or tetrazotized benzidine coupling technique (Barka and Anderson, 1965) for demonstration of protein or conjugated protein gave positive results, which indicates a mucoprotein or glycoprotein (PLATE 11). There appears to be no

TABLE I. Summary of Histochemical Reactions of Unicellular Glands of *Phoronis vancouverensis* Pixell.

				Results obtained with	
Name of Test	Fixation	Embedding medium	Reference	Basiphil type	Acidophil type
PAS	S;B;Z	P	Pearse (1959)	---	+++
PAS control	S;B;Z	P	Pearse (1959)	---	---
PAS after diastase	S	P	Pearse (1959)	---	---
PAS after acetylation	S	P	B & A (1965)	---	---
Sudan III, IV	fresh frozen		B & A (1965)	---	---
Ninhydrin	S	P	B & A (1965)	---	+++
Tetrazotized benzidine	S	P	B & A (1965)	---	+++
Alcian Blue	S;B;Z	P	Pearse (1959)	+++	---
Alcian Blue after hyaluronidase	Z	P	B & A (1965)	+++	---
Toluidine blue metachromasy	Z	P	B & A (1965)	+++	---
Methylene blue extinction point	Z	P	B & A (1965)	pH 3.5	---

Key: B = Bouin's fluid; B & A = Barka and Anderson; P = paraffin; S = Heidenhain's 'Susa' fluid; Z = Zenker's fluid; +++ = strong reaction; --- = no reaction.

histochemical technique capable of distinguishing between these two proteins.

The strong Alcian Blue reaction in the contents of the second gland cell type points to the presence of an acid mucopolysaccharide, hyaluronic acid, or chondroitin sulfate (Pearse, 1959). The staining reaction remained fast following hyaluronidase digestion. The methylene blue extinction point was found to be circa pH 3.6. Toluidine staining of these gland cells resulted in a strong gamma type metachromasia, meaning a color shift from blue to red. These various responses, especially the strong metachromasia, are considered characteristic of sulfated mucopolysaccharides (Barka and Anderson, 1965). The consistent stain reaction following hyaluronidase digestion indicates the presence of the chondroitin sulfate type B (Pearse, 1959).

The histochemical reactions of the unicellular epidermal glands are summarized in TABLE I. The reactions, in summary, clearly indicate the presence of two gland cell types. The first, acidophil, produces a small globular secretion of muco- or glyco-proteinaceous nature. The second, basiphil, produces an acid mucopolysaccharide, probably a highly sulfated ester similar to the chondroitin sulfate type B.

E. Ciliary Feeding Mechanism

In the normal feeding position Ph. vancouverensis has the top one-third of its trunk free of its tube (FRONTISPIECE). The lophophore is fully expanded with the outer tentacle ring extended, while the tentacles of the inner tentacle ring are closely 'bunched' together.

The feeding apparatus is comprised of four fully integrated systems: i. current-producing; ii. particle-collecting; iii. particle-sorting; iv. particle-rejecting.

i. current-producing. The lateral cilia produce the filtration current through the lophophore by their continuous oral to aboral beating. This draws a continuous downward flow of water between the tentacles. Thus an in-current enters the lophophore chamber from above, carrying with it any particles suspended in the water (FIG. 3). The exit-current, produced by these same cilia, passes through the outer tentacle ring in a downward direction, and through the inner tentacle ring into the excurrent chamber between the arms of the lophophore (FIGS. 1B, 3). From the excurrent chamber the water current passes in an upward direction over the anus and nephridiopores, exiting between the arms of the lophophore.

Water currents, entering the lophophore chamber near the mid-line, pass straight down into the buccal groove, carrying particles down toward the mouth. These currents then exit either through the outer or the inner tentacle ring as described above. Currents entering the arms of the lophophore have two alternate directions. Either the current passes straight down into the lophophore chamber, there joining the main buccal groove current toward the mouth, or the current is swept away from the direction of the mouth into the extreme ends of the lophophore arms. The latter can be explained by the fact that the tentacles in the extremities of the lophophore arms are closer together, enclosing much less volume, thus creating a very strong exit-current via the lophophore arms (FIG. 3).

The currents within the actual buccal groove are complex. The buccal groove is widest (circa 350 μ)at the mouth, and narrowest (circa 100 μ) at the extreme ends of the groove in the lophophore arms (FIGS. 1A, 4). With the in-current flowing continuously down into the buccal groove, and with the buccal groove being completely enclosed by the fused bases of the outer and

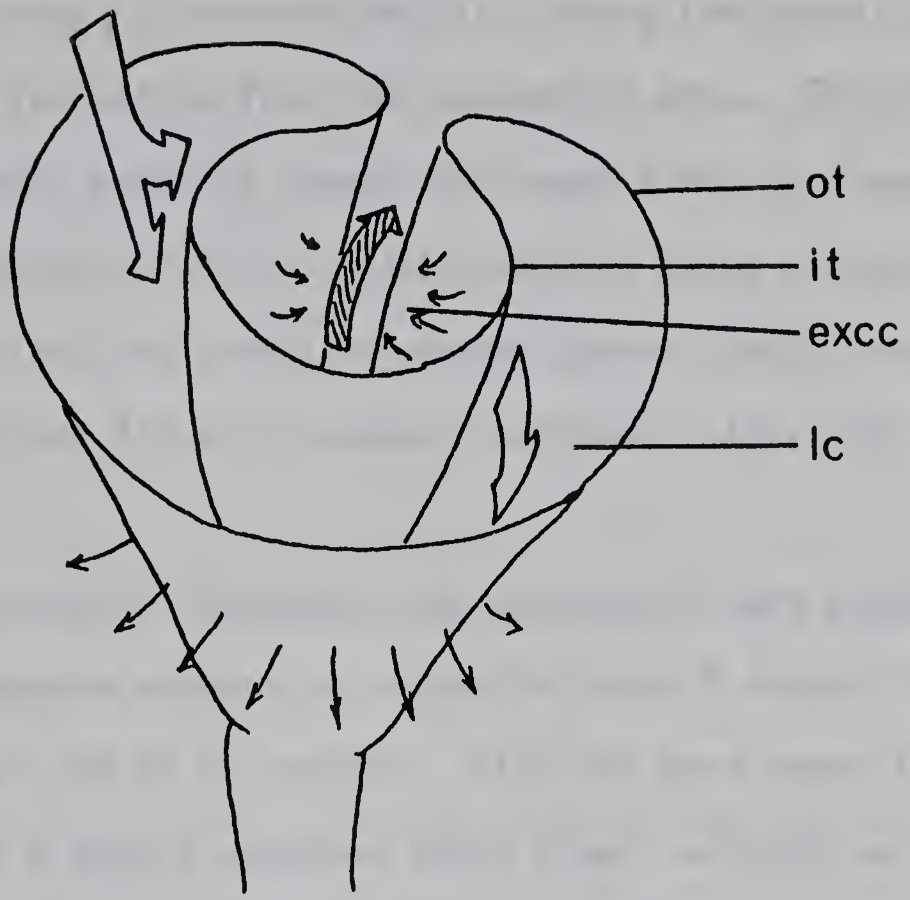


FIGURE 3. Schematic representation of the lophophore, showing in-currents (white arrows) and exit-currents (black arrows). excc, excurrent chamber. it, inner tentacle ring. ot, outer tentacle ring. lc, lophophore chamber. X 60

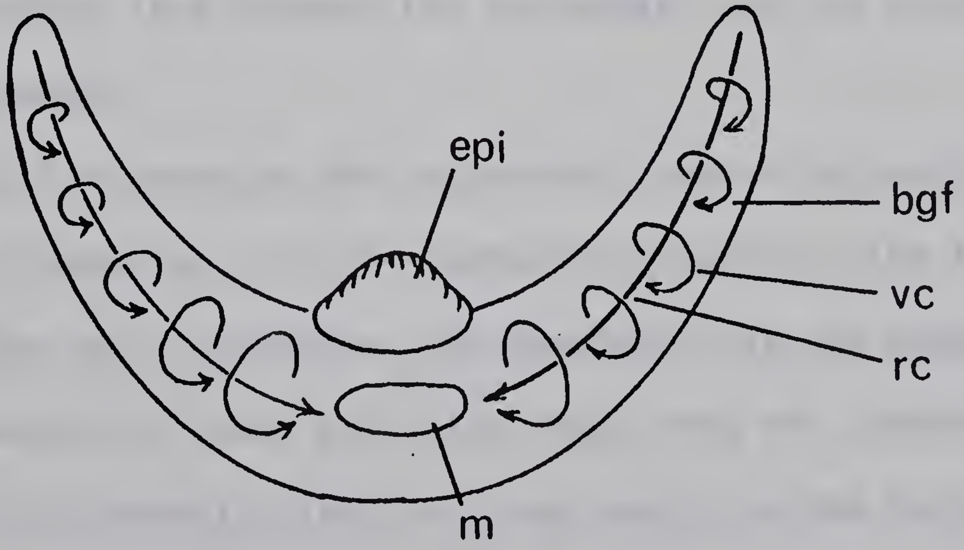
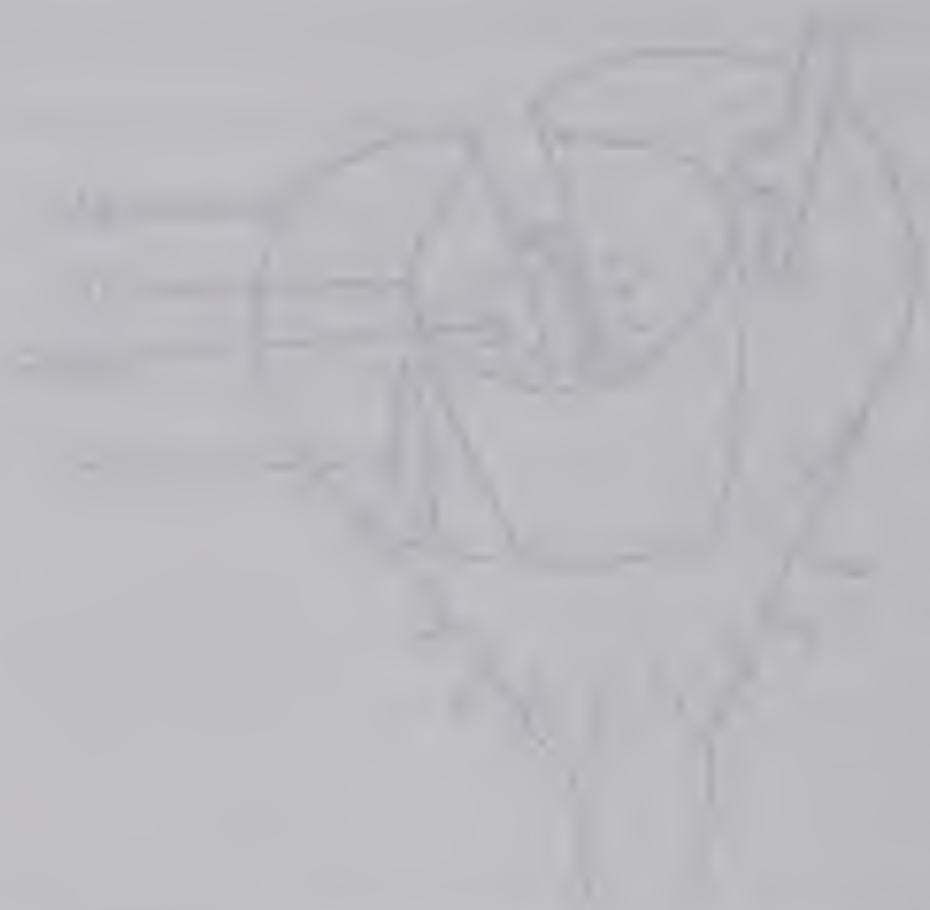


FIGURE 4. Schematic representation of current patterns in buccal groove. Bgf, buccal groove floor. epi, epistome. m, mouth. rc, resultant buccal current. vc, vortex current.



[illegible]

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inner tentacle rings, a vortex is created medially along the buccal groove by incoming high current velocities from the lophophore arms. The net result is a current directed medially toward the mouth along the length of the floor of the buccal groove (FIG. 4). The epistome, lying dorsal to the mouth, acts in deflecting the resultant buccal groove current into the mouth. Excising the epistome did not, however, seriously alter the current patterns.

Water current velocities throughout the lophophore vary greatly. Particles enter the lophophore chamber at a rate of about 8 cm/sec. Exit-currents may go as high as 100 to 150 cm/sec. With the open upper feeding area of the lophophore of a mature specimen about 9 mm^2, as much as 2 to 2.5 liters of seawater can be carried through the lophophore per hour by the beating of the lateral cilia.

ii. particle-collecting. Only those particles above the lophophore figure in feeding; those particles lateral to the lophophore are in the path of the exit-current coming from between the tentacles, and are swept away from the lophophore chamber.

Of those particles entering the lophophore chamber the majority are swept out between the tentacles. Of the particles swept out, the larger number exit between the outer tentacles, the remainder via the inner tentacle ring. Less than a quarter of those particles swept into the lophophore chamber are estimated to actually find their way down into the buccal groove and to the mouth.

Particles descended into the lophophore chamber and buccal groove on water currents only. At no time were particles observed being carried to

the mouth by ciliary pathways on the tentacles. Tentacles, however, do play a major role in particle transport. When the animal is feeding, the lophophore is spread out to an angle of about 45° at its base (FRONTISPIECE). Individual tentacles are constantly in motion, flicking particles bouncing against their oral surfaces into the lophophore chamber. The tentacles do not envelop a particle and carry it actively to the mouth, but when hit by a small particle on the in-current, either the whole tentacle or just the tip will flex quickly, causing the particle to fall more deeply into the lophophore chamber.

iii. particle-sorting. Those particles reaching the buccal groove are mechanically sorted into two categories: those over 250 to 300 μ, and those under. In sagittal section the lophophore chamber is funnel-shaped (FIG. 1B) with the mouth at the lowest point. Particles over 300 μ are either too large to fall completely to the bottom of the lophophore chamber, or are deflected by the epistome. These are then carried upward along the oral faces of the tentacles by the frontal cilia up to the upper rim of the lophophore, where they are 'dumped' into the exit-current. This ejection pattern appears constant. Rejected particles are carried up the tentacle by the frontal cilia until they either fall off into the exit-current, or they are carried to the tentacle tips where they are then rejected into the exit-current.

The current exiting through the inner tentacle ring and passing out over the anus and nephridiopores is directed upward and away from the lophophore chamber (FIG. 3). In this manner, fecal pellets which might otherwise fall back into the lophophore chamber are carried away from the

TABLE II. Content Analysis of Fecal Pellets from *Ph. vancouverensis* Pixell in different localities, and of ambient seawater samples.

Taxa	Laboratory seawater system *	Fecal pellet *	Friday Harbor Bay*	Fecal pellet *	Westcott Bay *	Fecal pellet *	size (microns)
Coscinodiscus sp.	< 1 %	< 1 %	10 %	10 %			70-200
Skeletonema sp.	95	95	50	50	90 %	90 %	10-30
Navicula sp.	< 1	< 1	20	20	< 1	< 1	20
Fragillaria sp.	< 1	< 1	10	10			50
Tintinopis sp.					< 1	< 1	
Trachyneis sp.	< 1	< 1	10	10			100
Cosconeis sp.							10-30
green flagellates							20
silica flagellates							100
Chaetocorus sp.					4	2	
Thallasiosira sp.					2	4	
Ditilum sp.						< 1	
sand grains			10	5	< 5	< 10	100-200

*Percentage composition of sample. Sample size: fecal pellets - 40, seawater - 1500 ml. Results based on phase-contrast microscopic examination, identification of plankton based on Gran *et al.* (1931) and Cupp (1943).

direction of the mouth. In mature Ph. vancouverensis the larvae are brooded on special lophophoral organs within this inner excurrent chamber (Zimmer, 1967). When the larvae reach the free-swimming pelagic stage they are released into the sea in the exit-current away from the lophophore chamber and the mouth.

Apparently particle sorting is non-qualitative. Under laboratory conditions approximating natural conditions the organisms readily accepted all particles under 300 μ in diameter introduced into the buccal groove, including small fecal pellets and immature non-motile larvae. A microscopic analysis of fecal pellets taken from clusters of specimens reared in different localities as compared with particulate content of respective ambient seawater samples showed the particulate compositions to be similar (TABLE II).

iv. particle-rejecting. The ejection of oversized particles via the frontal cilia to the lophophore rim has been discussed above. Frequently particles ejected via the lophophore arms. The close proximity of adjacent tentacles in the lophophore arm extremities (FIG. 1A) provides for a relatively uninterrupted area of frontal cilia. This together with the high exit-currents from the lophophore arms provides for a highly effective ejection mechanism.

An excessively high concentration of particles on the lophophore impedes the operation of the ciliary mechanisms. On some occasions, as when silt is carried into the lophophore of a feeding specimen, several adjacent tentacles bend outward, thus allowing the surplus debris and particles to be spilled out through the so-formed gap in the tentacle ring.

Such cooperative ejection appears not to be the function of definite groups of tentacles, nor of a certain minimum or maximum number of tentacles. Any region of the lophophore can be induced to this ejection activity by simply loading that portion of the buccal groove with excess particles.

An interesting ejection behavior is exhibited when, infrequently, a fecal pellet falls back into the lophophore groove. The pellet, after mechanical sorting in the buccal groove, is either ejected via the frontal cilia on the outer tentacle ring, or more complexly via the inner tentacles. If ejected via the inner tentacles, the fecal pellet is first carried to the tentacle tips of the nearest four or five tentacles by their frontal cilia, where the pellet is then passed from tentacle to tentacle toward the lophophore arms and ejected. In this process, the tentacle tips are very closely applied to the pellet, and often appear to be nearly wrapped about it. This behavior can be elicited at any time by introducing any long thin particle to the base of the inner tentacle ring, on each occasion the nearby tentacles carrying the particle along their tips toward the nearest lophophore arm for ejection.

F. The Role of Mucus

No mucus film was observed over the surfaces of the tentacles. Nor was there evidence of mucous webs or other mucous particle trapping over the lophophore. Only particles deep in the buccal groove appeared coated with mucus.

G. Discussion

It appears from the results of this study that the feeding mechanism as exhibited by Ph. vancouverensis is not as that described for the phylum

by Hyman (1959), i.e. ciliary-mucus feeding. This generally accepted description is based on earlier descriptions by Gilchrist (1907) of *Ph. capensis* and Cori (1939) of *Ph. viridis* and *Ph. hippocrepia* that cilia on the tentacles actively carry food particles to the mouth "wie auf einem laufenden Band" (Cori, 1939). In fact, the frontal cilia beat from the base of the tentacle to the tip, and reversal of this pattern, has not been observed. Thus the frontal cilia act only as a mechanism for rejection of particles. This explains Gilchrist's observation that particles "after reaching the base of the tentacles, are being returned on the same tentacles, and seen travelling as rapidly in the opposite direction to the distal end of the tentacle". *Ph. vancouverensis*, in feeding, apparently relies solely on particles being carried by water currents into the bottom of the lophophore chamber toward the mouth. Ciliation in the buccal groove aids in creating currents which then propel particles into the mouth.

Gilchrist (1907) attempts to explain the apparent reversal of particle transport on the oral face of the tentacle by suggesting that the lateral cilia beat in opposite directions, and that the tentacle by rotation about its long axis can present either an upward beating or downward beating ciliary tract depending on which side of the tentacle is turned into the oral chamber. The situation is, in fact, much simpler than earlier described. With phase-contrast microscopic examination of whole lophophores and of excised portions of lophophores, it is clear that the lateral cilia on both sides of the tentacle beat at right angles to the long axis of the tentacles, in an oral to aboral direction. Their metachronal beating creates an incoming water current from above the lophophore, drawing it into the

lophophore chamber, and driving it outward and downward through the intertentacular spaces. The current patterns in Ph. vancouverensis are as those described for Ectoprocta (Atkins, 1932) and Brachiopoda (Atkins and Rudwick, 1962), but are unlike those described for the Endoprocta (Atkins, 1932). Thus, in Phoronida, Ectoprocta, and Brachiopoda the lateral cilia act only in setting up the necessary water currents, and do not directly transport particles. Dawydoff et al (1959) write that the method of feeding is apparently similar to that of the Lamellibranch Molluscs, i.e. carrying mucus entrapped particles to the mouth by means of ciliary pathways. This, for the reasons outlined above, does not hold true for Ph. vancouverensis. Mucus, except for coating particles deep in the buccal groove and for possibly a minor role in entrapping particles in the buccal groove, does not appear to act in trapping particles in the sense of mucous films or webs over the tentacles.

Nothing is known about the specific role of the latero-frontal cilia. While the frontal and lateral cilia have definite beating patterns and specific functions, the latero-frontals beat only infrequently, individually, and in no definite direction. Such latero-frontals are described for Lamellibranchs (Jørgensen, 1954) where they are seen as coarse intermediates between the lateral and frontal cilia, but no specific role is ascribed to them.

Contrary to opinions held by Hilton (1922) and Clark (1964), tentacle movement in a well-coordinated fashion in Ph. vancouverensis is very pronounced. Tentacles move both singly and in groups. On many occasions tentacles were observed bending outward, either individually or in conjunction with several adjacent tentacles, creating a gap in the tentacle ring, allowing

spilling over of excess particles. The tentacles of the lophophore are well innervated, each tentacle receiving a nerve from the anterior nerve ring underlying the outer ridge of the lophophore (Hilton, 1922; Silèn, 1954).

The lophophore as a filter feeding device is an inefficient structure. Less than half of the current-born particles entering the lophophore chamber reach the mouth. The energy requirements of the animal are met, however, by movement of large amounts of water, an estimated 1 to 1.5 liters of seawater being carried through the lophophore per hour. No values were found available in the literature for amino-nitrogen content of the animal in order to convert the filtration rate per hour to one based on nitrogen content, as has become standard (Jørgensen, 1949, 1954; Carlisle, 1966).

Ph. vancouverensis does not appear to qualitatively select food particles. The comparative analysis of fecal pellets and ambient seawater from three different localities (TABLE II) clearly shows that the types and proportions of particles in both samples are similar. This suggests that selection of particles by size is the only means used by this organism to discriminate among the particulate matter carried into the lophophore chamber on the in-currents.

The possibility of chemosensitivity and of increased feeding activity induced by the presence in the seawater of certain dissolved substances, ions, or metabolites of course exists. Such behavior and chemosensitivity are reported for the sea star Patiria miniata (G. S. Araki, 1964, personal communication). This aspect of feeding behavior was not explored in this study.

Histochemical results, as summarized in TABLE I, clearly confirm the histological separation of epidermal unicellular glands into two chemically different types. The first, basiphil, found in large numbers in the trunk epidermis and the buccal groove epithelium, secretes a highly sulfated ester, resembling chondroitin sulfate-B, as a film over the trunk of the organism (PLATE 11). Presumably this is of a mucoid nature. The second gland cell type, acidophil, secretes a muco- or glyco-proteinaceous product, and is found in large numbers over the aboral faces of the tentacles. Its function is not known.

A comparison of these present findings with those of earlier workers if anything renders the situation more confusing, rather than resolving existing uncertainties regarding the gland cells and their secretions. Except for Pixell (1922), who describes three gland cell types in Ph. vancouverensis, there is general agreement on the presence of two gland cell types (Selys-Longchamps, 1907; Cori, 1939; Marcus, 1949; Silèn, 1952; Hyman, 1959). They are described respectively as acidophil and basiphil. Marcus (1949) and Silèn (1952) disagree, however, as to which is basiphil. Furthermore, where Marcus (1949) observes that the basiphil cells in Ph. ovalis secrete a substance dissolving the calcareous substrate, Silèn (1952) sees these cells in Ph. pallida as secreting the tube. While the present findings agree with Silèn (1952) on this point, the histochemical results outlined above, and in TABLE I, do not support Silèn's findings that their secretion is proteinaceous, as indicated by their strong reaction to protargol (Conn, 1961). On the contrary, the present results clearly show a non-proteinaceous content of the basiphil cells, and a very positive protein content as shown with ninhydrin or benzidine in the

acidophil gland cells (PLATE 11). Whether these apparent discrepancies are due to species differences, or to fixation and embedding techniques is not known.

Light and electron microscopy further confirm the presence of two distinct epidermal gland cell types. The basiphil mucus secreting cell is typified by a compacted mass of globules, increasing apically, similar in appearance to goblet cell contents described for intestinal epithelia (Hollmann, 1965; Freeman, 1966). The acidophil gland cell of the tentacles and trunk is found to contain large numbers of small droplets, frequently distending the cell volume, when seen by light microscopy in paraffin sections. Electron microscopy observations do not entirely support this, fewer numbers of globules being found than expected from light microscopic studies. The secretion after discharge of the acidophil gland cells appears as small globules, about 4000 Å in diameter, over the surface of the tentacle cells (PLATES 7, 8).

The many small circular profiles enveloped in a fine layer of coat material (PLATES 8, 9) probably represent different sections through multi-digitate microvillous developments of the apical cell membrane. Thus, the irregularly shaped structures (PLATE 9) found in some sections may represent axial elements of multi-digitate microvilli, while the small outbulgings of the distal plasmalemma seen in some sections may represent the bases of such microvilli (PLATE 5). The appearance of the coat material characteristic of these various structures is consistent with that described for absorptive regions of the intestine (Ito, 1965). It is also found over the small microvilli extending from some of the tentacle cells (PLATES 5, 6). This development of the external surface membrane into microvilli

is not uncommon. A dense brush border of microvilli has been described for non-ciliated secretory cells in the gill epithelium in Mytilus edulis (Afzelius, 1960). No statement about the role of these microvilli was made. It is possible that in Ph. vancouverensis these external microvilli play an absorptive role, allowing for direct transport of ions or metabolites from the ambient seawater. Such uptake of dissolved organic material has been described for sea anemones, polychaetes, and molluscs, even when the digestive tract had been blocked (Stephens, 1964). A more thorough electron microscopic study is necessary to give more substance to these findings.

IV. DIGESTIVE TRACT

A. Introduction

Most observations on the digestive tract and its functions in Phoronida are restricted to those of a morphological character (Becker, 1937; Cori, 1939; Marcus, 1949; Ohuye, 1942). The general structure of the gut tract has been described by the use of the light microscope, and in general, five regions are accepted as structurally and functionally distinct, namely, a short oesophagus, a proventriculus or pre-stomach, a stomach at the base of the descending gut loop and, in the ascending gut loop, the intestine and the rectum (FIG. 5). A detailed description of cell structure, secretory granules, intra-cellular digestion, and ciliation is recorded for the actinotroch larva by Becker (1937) who assumes that the observations for the larva are also valid for the adult phoronid. Hyman (1959) and Dawydoff _et al._ (1959) suggest an enzymatic character for the secretory granules described by Becker (1937).

The oesophagus is described as consisting of a highly ciliated columnar epithelium, containing numerous gland cells with alveolar or homogeneous contents (Silèn, 1952). There is some doubt as to the actual character of this epithelium, Silèn (1952) describing two layers of cells, a columnar layer overlying a basal layer of large globular cells which extend along the basement membrane. He concedes, however, that this may be a case of pseudostratification, observing that the more distal columnar cells "reach the basal membrane in the often very narrow interspaces between

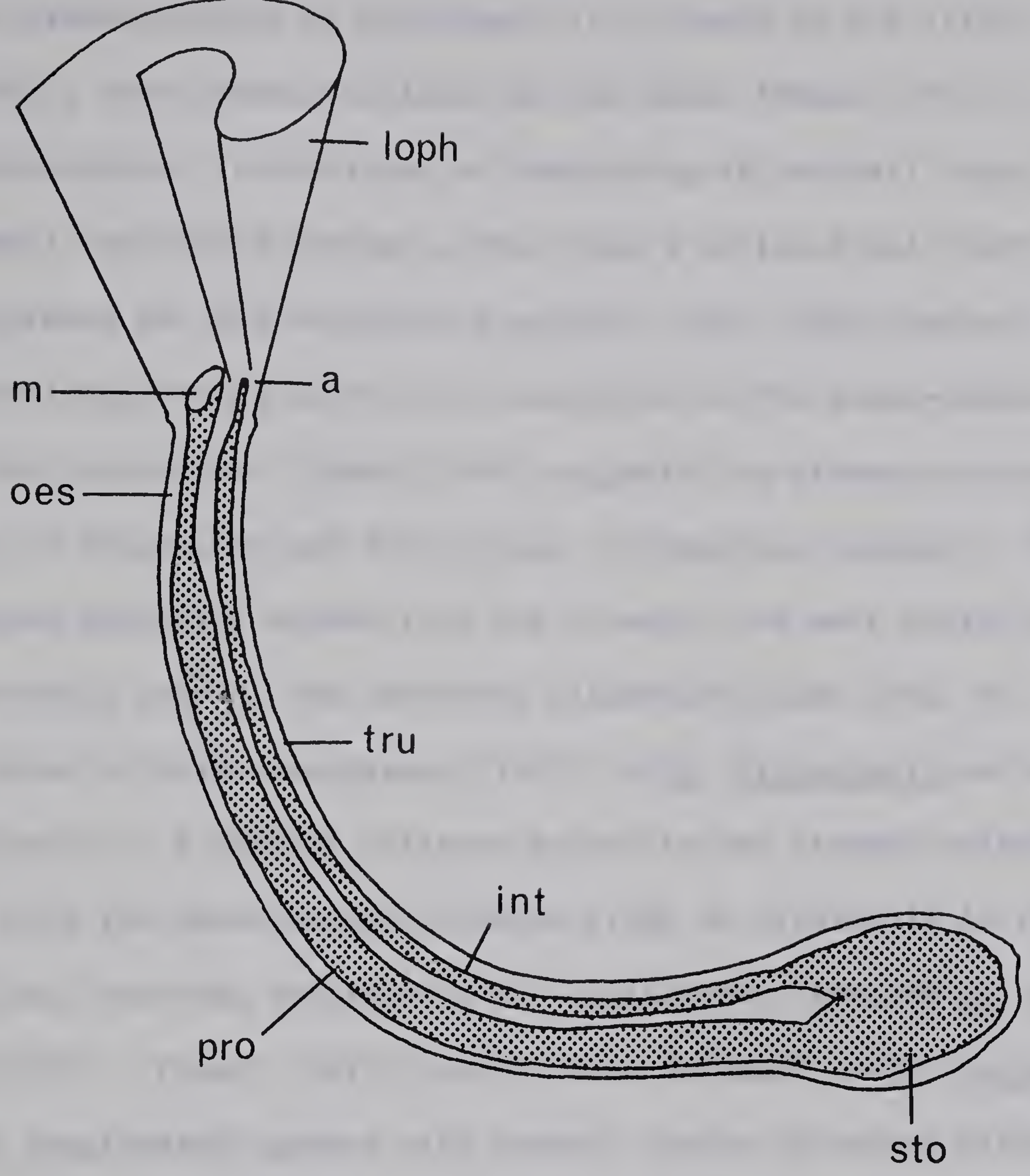

FIGURE 5. Schematic two-dimensional representation of the digestive tract in Phoronis vancouverensis Pixell. a, anus. int, intestine. loph, lophophore. m, mouth. oes, oesophagus. pro, proventriculus. sto, stomach. tru, trunk.

the globular cells". Hyman (1959) suggests that these apparent basal cells are in fact wandering coelomocytes. Numerous gland cells with alveolar content are found throughout this region. Becker (1937) writes of countless granular inclusions within the cell cytoplasm, probably of a secretory nature. He also reports vacuolated cells, resembling mucus cells found in annelids.

The proventriculus or prestomach is ciliated in the larva (Becker, 1937), less densely ciliated in the adult (Silèn, 1952). The larval proventriculus is described as consisting of two cell types, one with acidophil vacuolated contents, the other a ciliated cell with a brush border containing fat and secretory granules. Cori (1939) reports a ciliated typhlosole lying anterior to posterior in the dorso-median region of the epithelium. Hyman (1959) suggests the proventriculus acts as a region of absorption and for storage of digestion products. The proventriculus gradually widens into the stomach, the most distal portion of the descending loop of the recurving alimentary tract (FIG. 5). The latter is shown by Selys-Longchamps (1907) in Ph. hippocrepia as having a ciliated gutter. A similar ciliated gutter in the stomach epithelium, continuous with the dorso-median ciliated ridge or typhlosole in the proventriculus, has been reported in the actinotroch larva of Ph. ovalis by Becker (1937). Pixell (1912) describes the stomach of Ph. vancouverensis as having a longitudinal groove with several layers of nuclei staining "more deeply" than others, and with long cilia. She also records finding digestive areas "which carry on intracellular digestion, have no cilia, and in which the free ends of the cells are distinctly amoeboid during functional activity". She reports frequent rows of spherical deeply staining masses "probably oil globules", near the outer rows of the cells. Cori (1939) describes the stomach epithelium "losing its epithelial character and assuming a syncytial character". Such a syncytium has also been recorded by Becker (1937), and Silèn (1952). Some writers further report paired syncytial regions lying in the lateral walls of the stomach (Cori, 1939; Marcus, 1949; Silèn, 1952; Lönöy, 1954). These writers also describe

diatoms, and other undigested particulate matter lying within digestive vacuoles in the stomach epithelium (Cori, 1939; Marcus, 1949). Dawydoff *et al*. (1959) suggest that the syncytium in the anterior portion of the stomach (formed through incomplete fusion of the cell bases) appears capable of phagocytosis and subsequent intracellular digestion. They do not exclude the possibility, however, of extracellular section of enzymes and intra-luminal digestion of particulate matter.

The distal portions of the gut, the intestine and the rectum, are characterized in the literature by small cells, ciliated, often granulated (Lönöy, 1954), with possibly a secretory function. Becker (1937) describes a brush border on the epithelial cells of the intestine.

There is a paucity of literature and workers in the area of digestion in the phylum Phoronida. The reports of granular inclusions, syncytial composition of the stomach region, apparent holophagocytosis of entire algae and diatoms, absorption and storage in the proventriculus all need to be examined further. The whole question of intracellular or extracellular digestion and the relative importance of the two has been wanting since the first description of this phylum in 1846 by Müller. It is to this purpose that a combined histological, histochemical, and electron microscopic study was begun of the digestive tract.

B. Observations

i. Paraffin sections. The digestive tract of *Ph*. *vancouverensis* consists of a recurving digestive tract, with the oesophagus, proventriculus, and stomach regions in the descending loop, and the intestine and rectum in the returning ascending loop (FIG. 5). The two loops lie parallel to

one another in the trunk coelom, which is readily evident in trunk cross-sections (PLATE 15).

The buccal cavity and mouth in Ph. vancouverensis are lined by a simple columnar epithelium, densely ciliated with tall cilia (circa 8 to 10 μ), interspersed with numerous gland cells with globular contents. The epistome overlying the mouth is also densely ciliated, and particularly on its underside is furnished with numerous gland cells with Alcian Blue positive contents.

The oesophageal epithelium consists of tall simple columnar epithelium, densely ciliated, interspersed with gland cells with Alcian Blue positive contents. Scattered throughout the apical cytoplasm of the cells are small granules, staining red with PAS, or dark-brown with tetrazotized benzidine. The elongate nuclei lie basally in the cells. No evidence of a clearly defined ciliated gutter or groove was found. The entire periphery of the oesophageal lumen appeared uniformly ciliated.

The proventricular region of the gut is characterized by a simple tall epithelium with sparse ciliation. Gland cells containing Alcian Blue positive contents are less numerous than in the oesophageal epithelium. Small granules, staining positively with PAS (PLATE 16) or with tetrazotized benzidine, are scattered in large numbers throughout the super-nuclear regions of the epithelium. No foodgroove or ciliated gutter was discernible. The underlying basement membrane is featureless, lacking any cellular detail.

The stomach is much enlarged, both in tissue mass, girth, and lumen, relative to the other regions of the digestive tract. The epithelium consists of a dense mass of sparsely ciliated tall columnar cells. Under the light microscope it was impossible to determine whether the epithelium

was stratified or pseudostratified. No syncytial areas were observed, nor could a ciliated groove or tract be demonstrated. The contour of the lumen, however, is highly convoluted, giving the impression of specialized epithelial tracts. The cells contain numerous small granules, visible with PAS or tetrazotized benzidine staining, in their apical cytoplasmic regions. No diatoms or remains of their skeletons, or any particulate matter was found within the stomach epithelial cells.

The hindgut or intestine occupies most of the ascending gut loop (FIG. 5). Its anterior portion consists of tall epithelium, highly ciliated, with many convolutions of the lumen contour apparent in cross-section. No food groove was noted. PAS-positive granules are less numerous than in preceding gut regions. Gland cells with Alcian Blue contents were found scattered throughout the epithelium. The epithelium gradually changes to a cuboidal form in the hindmost regions of the intestine. The intestine is ciliated throughout its length, with oval nuclei consistently in the basal and median regions of the cells. In cross-section the intestine is considerably smaller in diameter (circa 100 μ) than the descending gut regions. No ciliated gutters, or regions of high ciliation were observed. Throughout the epithelium of the intestine lie scattered gland cells with Alcian Blue positive contents, in appearance similar to the mucus secreting cells of the trunk epidermis.

ii. Epon sections. Examination of thick and thin sections confirms that the epithelium of the buccal groove, mouth, and oesophagus is a simple columnar epithelium. The cells are tall, and at times very tortuously twisted with their basal portions often very thin, yet resting flatly on the underlying basement membrane. Two cell types are found in this region.

The first is a ciliated cell, the second contains a compacted mass of large globules, similar to mucus secreting cells found in the trunk epidermis and described elsewhere (Hollmann, 1965; Freeman, 1966).

The proventricular epithelium consists of simple columnar cells, resting basally on the underlying basement membrane. The region is uniformly ciliated, one cilium per cell. The nuclei are elongate (circa 2.5 μ wide and 7 μ long), are primarily found in the median or basal portions of the cells, and have homogeneous, electron-dense contents. Scattered throughout the cell cytoplasm are strands of endoplasmic reticulum, frequently exhibiting flat, saccular expansions approximately 0.5 μ in diameter (PLATES 17, 18). Free ribosomes are scattered throughout the cells. The Golgi complex generally consists of from four to six curved double membrane elements, enclosing an inner cavity roughly 0.25 μ in diameter (PLATES 19, 20). The distal tips of the elements are frequently distended into vacuoles about 1000 Å in diameter. Vesicles of varying sizes are intimately associated with the complex. The contents of some of these vesicles appear similar in electron density to the contents of the Golgi elements (PLATE 20).

Many single-membrane bounded spherical granules are found in the supra-nuclear regions of these cells (PLATES 17, 18, 21, 22). These granules measure up to 0.5 μ in diameter, and contain an electron dense homogeneous core. Lipid bodies are frequently found scattered throughout the cytoplasm of these epithelial cells (PLATE 23).

The surface cell membrane in this region is irregular, frequently elaborated into microvillous projections (PLATE 24). Small, about 0.1 to 0.2 μ, 'pinocytotic' vesicles are frequently found associated with the

distal cell membrane and the apical cytoplasm (PLATES 18, 25). Found frequently in such regions are multivesicular bodies, sometimes with myelin-like figures within their interior (PLATE 25).

The cilium of this region, in cross-section, displays the characteristic nine double peripheral and two single central fibrillar configuration. The cilium is characterized by a definite basal plate, primary and secondary basal bodies, main ciliary rootlet and a second shorter rootlet (PLATES 21, 22). The major cross-striation periodicity of the rootlet is about 850 Å. Two narrower cross-striations are discernible between the prominent bands delimiting the major period.

A few cells were found containing granules differing in appearance and size from the single-membrane granules described for the supra-nuclear cytoplasmic region. This second granule type is about 150 to 200 mμ in diameter, and has a very electron-dense core separated from the limiting single membrane by a clear space (PLATE 26). The granules lie densely scattered throughout the basal cytoplasm of these cells.

The overall appearance of the stomach epithelium is one of very tall and narrow columnar cells, containing a profusion of the single-membrane bound granules in the supra-nuclear regions, and an extremely dense scattering of rough endoplasmic reticulum, frequently in the form of dilated vesicles regularly arranged throughout the columnar cells (PLATE 27). Some cells are completely filled with extremely distended cisternae up to 0.3 μ wide and up to 1 μ long, arrayed in nearly parallel patterns. Free ribosomes and polysomes (PLATE 28) lie scattered throughout the cells, and are found attached to the outer membrane of the nuclear envelope. Elements of rough endoplasmic reticulum

and single membrane bounded granules are frequently found closely applied to large mitochondria (PLATE 29). The matrix of such mitochondria is transected by large numbers of plate-like cristae.

The Golgi complex in the stomach epithelium consists of from four to six curved elements (PLATES 27, 30, 31), of which the innermost elements are frequently distended with swollen tips. Numerous small vesicles are frequently accumulated within the cavity formed by the innermost concave elements. Single-membrane bounded granules are often found in close proximity to Golgi complexes (PLATES 30, 31).

Lipid bodies, up to 0.3 μ in diameter, lie scattered throughout the basal regions of the epithelial cells.

The stomach region appears uniformly ciliated. No clearly defined ciliated tracts or food grooves were seen. The epithelial surface is deeply infolded. In such infoldings the cilia stand very densely packed.

The anterior portion of the intestine is characterized by tall columnar cells, dense ciliation, and a highly convoluted epithelial surface. Electron micrographs of gut content in this region show a mixture of amorphous electron dense masses and of whorled configurations (PLATE 32). The single-membrane bounded granules found in large numbers in preceding portions of the gut epithelium occur in much reduced numbers in this anterior portion of the intestine (PLATE 33). Their general appearance, size, and density is similar to those previously described.

The anterior portion of the intestine is densely ciliated. No ciliated tracts or gutters _per se_ were discernible. The ciliary apparatus appears to consist of paired cilia, enveloped basally by a common membrane

(PLATE 34). Both members of the paired cilia exhibit the characteristic nine plus two configuration, with the plane of the central fibrils being the same in both cilia. The general structure of the cilia is similar to that described for the proventricular and stomach ciliation, in the primary and secondary basal bodies, and the striking cross-striation with a major period of circa 800 Å (PLATES 33, 35).

Large mucus secreting cells, their interior greatly dilated with the secretory contents, are found throughout the intestinal epithelium (PLATE 36). Such mucus cells occur singly, and in general appearance resemble the mucus secreting cells in the trunk epidermis (PLATE 12). They are characterized by a prominent distal mass of mucigen droplets and by the high electron-dense cytoplasm. The nucleus of these mucus secreting cells occupies a basal position.

Occasionally cells with a pronounced darker appearing cytoplasm are found in the intestinal epithelium (PLATE 37). The entire matrix of the cytoplasm as well as all the organelles within the cell appear darker than that of the surrounding cells. The mitochondria in such cells appear slightly wrinkled, and are slightly smaller in diameter than those of adjacent lighter cells. The dark cells are not shrunken, nor were unusual inclusions noted. They bear cilia, and do not appear obviously associated with such special functions as secretion or storage.

The posterior regions of the intestine are composed of nearly cuboidal epithelial cells. The region is ciliated, but without discernible ciliated tracts or gutters. The epithelium is highly infolded, displaying numerous folds in the epithelial contour of the lumen. The cells bear cilia singly, the internal structure of the cilia similar to that described

for ciliation in preceding portions of the digestive tract (PLATE 38).

Numerous microvilli project from the distal cell membrane of the epithelial cells into the lumen of the intestine (PLATE 39). The microvilli are very prominently enveloped within a finely fibrillar coat material. Numerous absorptive vesicles (up to 2100 Å in diameter) are found scattered throughout the apical cytoplasm of the epithelial cells (PLATE 40). Frequently a prominent layer of coat material is seen adherent to the inner side of the membrane limiting the absorptive vesicles.

Throughout the apical cytoplasm of the intestinal epithelial cells in a supra-nuclear position are found many single-membrane limited granules, up to 5000 Å in diameter (PLATES 39, 40). These granules exhibit a very electron-dense core of varying diameter, separated from the limiting membrane by a less dense region.

Small granules (up to 4000 Å) bounded by a single membrane were found in a few cells in a sub-nuclear position. The interior of these smaller granules is occupied by an intensely electron-dense core separated from the limiting membrane by a brief clear space (PLATE 41).

Lying basally in the intestinal epithelium, and in close proximity to the basement membrane, are numbers of large non-cellular masses enclosing numerous electron-dense spherical bodies (PLATE 42). These regions measure up to 11 μ in diameter. The intensely electron-dense spherical bodies (up to 1 μ in diameter) lie within individual 'pockets' bounded by membranous or myelin-whorl like configurations. None of the usual cell organelles as nuclei, mitochondria, or reticular elements are found in these regions.

iii. Enzyme and lipid histochemistry. Positive esterase demonstration in fresh frozen sections occurred at an optimum pH of 6.5 The test revealed that esterase activity is localized in small droplets (circa 1 to 1.5 μ in diameter), scattered throughout the supra-nuclear region of the epithelial cells (PLATE 43). Frequently only the periphery of the droplet was stained, the central core of the droplet remaining unstained. Esterase activity was found to be highest in the oesophagus and proventriculus epithelium (PLATE 43A), slightly less in the stomach, and very weak in the anterior portion of the intestine. No activity was noted for the remainder of the intestine.

Alkaline phosphatase reaction was found optimum at a pH of circa 8 to 8.5. As in the test for esterase activity, alkaline phosphatase activity was localized in small droplets scattered throughout the epithelia of the descending gut loop, primarily in the supra-nuclear region of the cells (PLATE 44). High magnification examination of the stain reaction showed that the peripheral boundary of the droplets stained most intensely. Staining density was most intense in the proventriculus, and strong in the oesophagus and stomach. No alkaline phosphatase activity was observed in the intestine.

Optimum demonstration of acid phosphatase occurred between pH 3.45 and pH 4.1. Again, the staining reaction occurred primarily in the perimeter of small droplets (about 1 μ in diameter) lying in a supra-nuclear position within the cells (PLATE 45). Reaction density was strongest in the proventricular and stomach epithelium, mild in the oesphageal epithelium. No or very weak reaction was noted in the intestine.

Sudan IV staining for lipid in fresh frozen sections yielded

positive reactions throughout the descending gut loop. Large numbers of Sudan IV positive globules (about 1 to 1.5 μ in diameter) were visible in the supra-nuclear regions of the epithelial cells of the oesophagus and proventriculus (PLATE 46). Scattered globules also were evident in the remainder of the cytoplasm of these cells. Sudan IV uptake in the stomach epithelium was less intense. No uptake of the stain was demonstrated for the intestinal epithelium. The stain colored the entire contents of the globules, but the periphery of the globules stained more intensely.

iv. Starch gel electrophoresis. Examination of the 'gel' strips following starch gel electrophoresis of extract from *Ph. vancouverensis* revealed the presence of seven esterases. Of these, bands 1 and 6 are very prominent (FIG. 6A). Bands 5, 6, and 7 did not appear in gels run with butanol treated extract.

Only one acid phosphatase could be separated from gut extracts by electrophoretic means. Even after a brief incubation time the staining intensity of this single band is very strong (FIG. 6B). Two strong and possibly one very weak band of alkaline phosphatase were demonstrated (FIG. 6B). Their mobility in the gel relative to the mobility of the acid phosphatase was low. Difficulty was encountered in obtaining consistent results among a series of trials for this enzyme.

Three, possibly four, proteases were electrophoretically separable in the enzyme complement of this organism. Where such bands appeared in the agar-starch gel overlay, digestion was complete (FIG. 6C). Bands 1 and 4 gave intense reactions, but band 2 did not consistently appear in several of the gels.

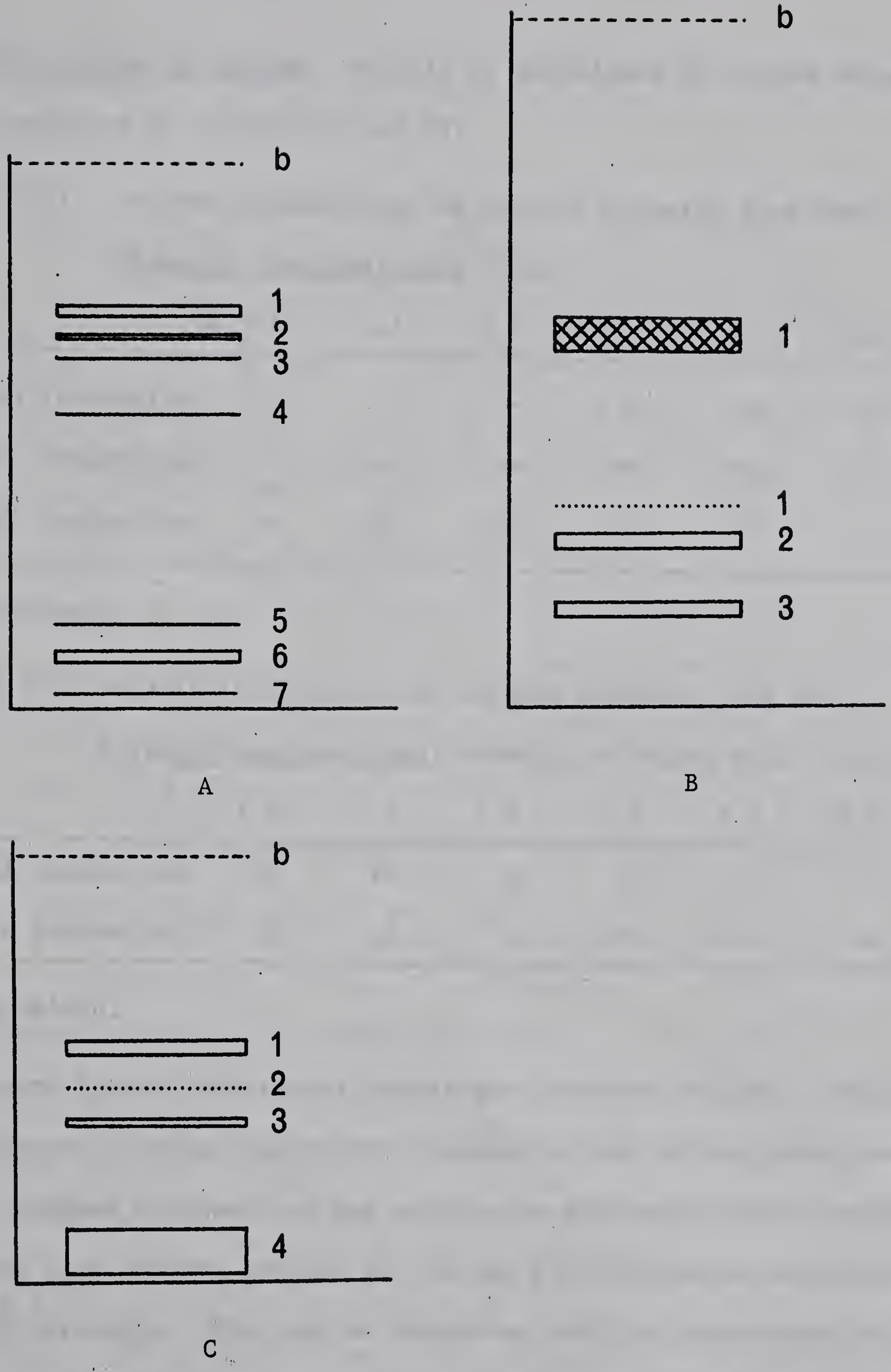

FIGURE 6. Zymograms of Phoronis vancouverensis Pixell. A, esterase. B, acid phosphatase (checkered) and alkaline phosphatase. C, gelatinase. b = position of moving boundary at termination of run.

[illegible]

v. Gelatinase pH optima. Results of gelatinase pH optima determinations are summarized in TABLES III and IV.

TABLE III. Gelatin Liquefaction by Extract prepared from whole *Phoronis vancouverensis* Pixell.

	pH 3.5	4.5	5.5	6.5	7.5	8.5
15 min incubation	0	+	++	++	++	++
30 min incubation	+	++	++	+++	+++	+++
60 min incubation	++	+++	+++	C	C	C

C = complete.

TABLE IV. Gelatin Liquefaction by Extract prepared from whole *Phoronis vancouverensis* Pixell, activated with Cysteine.

	pH 3.5	4.5	5.5	6.5	7.5	8.5
10 min incubation	+	++	+	0	+	0
30 min incubation	C	C	C	++	+++	++

C = complete.

pH optima varied considerably among the different trials. Cysteine activation, however, clearly showed the presence of two strong gelatinases; one with a pH optimum of about 4.5 and subject to activation with crysteine, the second with a pH optimum between pH 7.5 and 8.0, showing no sensitivity to addition of crysteine. The rate of digestion with the charcoal-gelatin technique was very rapid, even in very dilute solutions of the extract.

vi. Amylase spot test. Amylase was shown to be present in the extract prepared for electrophoresis. Only one pH optimum (pH 4.0) was detected.

C. Discussion

Morphology and Digestion:

The electron microscope has made possible a more thorough examination of details left doubtful with the earlier methods. Thus, the apparent stratified epithelium observed in the oesophagus by Silèn (1952) is probably due to oblique sectioning through basal portions of the oesophageal columnar cells, giving the appearance of slightly globular cells underlying a tall columnar epithelium.

No evidence was found for definite ciliary tracts or food grooves in Phoronis vancouverensis Pixell. Instead, particulate matter appears to be passed down the digestive tract by the epithelial cilia of the gut. This ciliation is uniform around the periphery of the lumen. Where a section was cut through an epithelial fold an apparent ciliated tract occurs. This, however, is only the result of the plane of the section.

The oesophageal portion of the descending gut loop appears to function primarily as the region of food ingestion and conduction, as indicated by the dense ciliation, the absence of microvilli, and the numerous unicellular glands throughout the epithelium. Histochemistry of the gland cell contents shows these to be of the same type as the basiphil mucus secreting cells in the trunk epidermis and the buccal cavity, i.e. probably a chondroitin sulfate type B (Pearse, 1959).

Unicellular mucus secreting glands are found throughout the digestive tract, increasing in numbers toward the posterior regions of the tract. Their total distribution appears not to be as great as described

for the actinotroch larva (Becker, 1937). The fine structure of these intestinal mucus cells seems similar to descriptions of intestinal mucus cells by Palay (1958) and others (Hollmann, 1965; Freeman, 1966).

Histochemical study of the proventriculus has shown this region as the site of strong esterase and phosphatase activity, localized in films overlying small droplets (circa 1 to 1.5 μ in diameter) (PLATES 43, 44, 45). Subsequent staining of fresh frozen sections of the proventriculus with Sudan IV showed lipid droplets (circa 1 to 2 μ in diameter) in large numbers within the epithelial cells (PLATE 46). The pattern of occurrence of the lipid droplets is similar to the small droplets showing positive enzyme activity in the form of 'enzyme films' surrounding a non-staining core.* Such films overlying fat droplets have been described for certain Lamellibranchs (Reid, 1965). This agrees with observations of George (1952) in Lamellibranchs and Cardell _et al._ (1966) in mammals that small fat droplets are found in the gut epithelial cells. The presence of enzyme films enveloping intracellular lipid droplets suggests that lipid metabolism may occur in the epithelial cells of the proventriculus. The fact that in electron micrographs lipid bodies do not occur in the same plenitude as demonstrated in fresh frozen sections may well be due to, firstly, loss of lipid in the preparative processes for electron microscopy (as has been demonstrated in guinea pig tissue up to 66.9%, Morgan _et al._, 1967), or secondly, to abnormal aggregation of smaller scattered lipid droplets concomittant with tissue and cell disruption in the preparation of frozen sections. A third possibility is that specimens used for the enzyme and lipid histochemistry were collected in the field in late spring (May), while those prepared for electron microscopy were collected in

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*No fat extraction was done prior to enzyme histochemistry, thus there is the possibility of artifacts due to slight solubility of the α-naphthyl substrate-azo dye complex in tissue lipids.

the fall (September), which seasonal difference may account for the fewer numbers of lipid bodies seen with electron microscope.

Light microscopic examination of the supra-nuclear cytoplasm of the proventriculus and stomach epithelia reveals a dense population of small granules (circa 1 to 1.5 μ in diameter). These granules stain positively with PAS or with a protein stain as ninhydrin or benzidine (PLATE 16). Electron micrographs of the same regions reveal a scattering of numerous single-membrane bounded granules (PLATES 17, 27), corresponding in size, numbers, and density to the PAS-positive granules. The general appearance of these granules is consistent with zymogen granules described by Palade (1959, 1961) and Fawcett (1966). The presence of large amounts of rough endoplasmic reticulum suggests that these granules originate in the reticulum in the form of secretory granules, thence presumably secreted into the lumen of the gut. No evidence has been found, however, of these granules being secreted. Since the organisms were maintained under artificial laboratory conditions, they may have been in a starved state. Under starved conditions there is evidence in the guinea pig (Palade, 1959) that secretory cells contain unusually large numbers of zymogen granules, but that none are found actively secreted into the gut lumen. Further work, using tracer techniques and autoradiography, is necessary to throw more light on these very introductory findings.

The stomach epithelium was observed consisting of tall thin columnar cells. No evidence of a syncytium was found as previously reported (Becker, 1937; Cori, 1939; Lönöy, 1954). The stomach epithelium is extremely convoluted and deeply infolded (PLATE 46). Deep folds so formed may well be the digestive pockets and large vacuolated regions

reported containing masses of diatom skeletons (Cori, 1939; Marcus, 1949), or the "great vesicles containing diatoms and other unicellular algae" (Lönöy, 1954). The stomach appears to act primarily as the center for mixing enzymes and foodstuffs. In normal feeding activity ingested food particles were observed to travel the length of the proventriculus in less than half a minute, subsequently remaining in the stomach for up to half an hour. This prolonged stay in the stomach would presumably insure thorough mixing of food particles with the various intra-luminal enzymes.

The appearance of certain gut contents (PLATE 32) suggests intraluminal breakdown of food materials. No electron microscope studies of gut content disintegration were found for comparison. Similar configurations have been obtained synthetically in suspensions of lipid complexes in water phases (Luzzati and Husson, 1962; Stoeckenius, 1962; Lucy and Glauert, 1964). The significance of such lamellar and myelin whorls is not known.

The high incidence of absorptive vesicles and microvilli in the intestine (PLATES 39, 40) suggests primarily an absorptive function. Digestion appears to be in the form of vesicles pinched off from the distal cell plasmalemma, and travelling inward into the cell cytoplasm. Some absorptive vesicles can be seen retaining a layer of coat material on the inner side of the limiting membrane, indicating a pinocytotic origin. The fate of these vesicles is unknown. The nature or role of the apical electron-dense structures found in this absorptive epithelium is not known. Their appearance, density, and numbers is not similar to the secretory granules described above for the proventriculus and stomach epithelia. It is possible that these are intermediate or residual stages in absorptive vesicle degradation.

The smaller granules, with an electron-dense central core, described for a few epithelial cells of the proventriculus and the intestine, appear similar to granules found in the Kultschitsky or argentaffin cells reported in the alimentary canal of certain mammals (Christie, 1955; Deschner, 1965; Hardmeier et al., 1965; Fawcett, 1966), or to the alpha-granules found in pancreatic cells (Fawcett, 1966). There is also a resemblance between these granules and neurosecretory granules described in both vertebrate and invertebrate tissue (Robertis, 1964). Absolute identification of these granules requires further, more intensive, histochemical study.

The general appearance of the large non-cellular regions lying in close proximity to the basement membrane in the intestine suggests a degenerate character, but is not consistent with the degenerative structures generally associated with focal degradation (Swift et al., 1964). The total absence of myelin whorls or of any intermediate stages point to some other sequence of events in this case. These regions were found in the intestinal epithelium of every specimen examined. The fact that these masses occur in the intestine may possibly suggest a connection between them and the absorptive character of this digestive region.

'Dark' cells, similar to those described above, have been reported scattered throughout parenchymal liver cells following administration of carcinogens to rats (Steiner et al., 1963), in inflammatory lesions in the human intestine (Shnitka, 1964), and among dog liver cells following ligation of the portal vein (Gansler, 1962). The increase in general electron density is postulated to be due to a state of relative dehydration of the cytoplasm and nucleus, or to an increased content of lipoprotein or phospholipid material in the cell (Shnitka, 1964).

Enzyme analysis:

The 'main' classes of digestive enzymes, i.e. carbohydrases, esterases, phosphatases, and proteases have been demonstrated for *Ph. vancouverensis*. The esterases consist of two main groups. The first group is made up of four isozymic forms (FIG. 6A). The second group, which advances less rapidly through the starch gel, consists of three isozymic forms and is labile in butanol. It is possible that these three esterases are particularly concerned with lipid digestion, while those of the first group (bands 1 to 4) are involved in other ester hydrolysis. This appears supported by the apparent slow mobility of bands 5, 6, and 7 in the starch gel, expected if these isozymes are physically or chemically bound to a lipid moiety. This possibility is further supported by the observation that butanol, in which lipids are soluble, eliminates the esterase activity of bands 5, 6, and 7.

Acid phosphatase activity was demonstrated in only one electrophoretically separable form. Its activity, under the conditions employed here, was very rapid and intense.

Extreme difficulty was encountered in obtaining consistent results in alkaline phosphatase demonstration. Whether these results, therefore, are valid is not known.

No attempt was made to histochemically localize protease activity in the tissue. The standard technique for the demonstration of L-leucine-amino-peptidase (Nachlas *et al*., 1957) has subsequently been invalidated by Patterson *et al*. (1963), who demonstrate that the substrate generally used in this technique (L-leucyl-β-naphthylamide) is hydrolyzed by several other enzymes as well. Two gelatinase types have been demonstrated present

in the extract, the first with a pH optimum of circa 4.5 and readily activated by cysteine, the second with a pH optimum of 7.5 and insensitive to cysteine activation. No cysteine activation studies were done on the gelatinase bands separated electrophoretically.

Carbohydrase investigation was limited to the demonstration of an amylase by use of a spot test outlined earlier. Amylase was not directly localized in the tissue, but it was shown to have a pH optimum of about pH 4.0.

Due to the minuteness of the organism whole animal extracts were prepared and used for electrophoretic separation of the enzymes generally associated with digestion. As a result, it is impossible to define the location of specific isozymes in specific digestive-tract regions. Such regional isozyme differences have been demonstrated for Lamellibranchs (Reid, 1965).

To date, the only other starch gel electrophoretic study of invertebrate digestive enzymes appears to be that of Reid (1965), although earlier data are available on vertebrate tissue (Uriel, 1960; Hunter and Markert, 1957). The presence of up to 14 esterases, 8 acid phosphatases, 4 alkaline phosphatases, and 8 gelatinases has been demonstrated by Reid (1965) in the two Lamellibranchs, Lima sp. and Mya sp. The comparative significance of the large numbers of the various digestive enzymes in the Lamellibranchs examined, and the relatively few numbers found in Ph. vancouverensis is not understood. Insufficient data are available, and too little is known about the digestive processes in this phylum to attempt any conclusive interpretations of this preliminary study. It must suffice at this time to state that the main 'digestive' enzyme groups have been shown

present and to some extent are characterized in extracts prepared from this animal.

V. SUMMARY AND CONCLUSIONS

The statement of Hyman (1959) that the Phoronida are ciliary-mucus feeders, based on earlier descriptions by Gilchrist (1907) and Cori (1939), has been re-examined in Phoronis vancouverensis Pixell. Feeding was found to consist of filtration of particulate matter from the ambient seawater. Water currents are drawn through the lophophore and particles are carried to the mouth on currents produced by the metachronal beating of cilia on the lateral faces of the lophophore tentacles (FIG. 2). The current patterns are found to be similar to those described for the other lophophorate groups, the Ectoprocta (Atkins, 1932) and Brachiopoda (Atkins and Rudwick, 1962). The frontal cilia do not play any part in the transportation of particles to the mouth. Rather, they are an important part of an ejection mechanism, carrying rejected particles from the buccal groove to the tip of the tentacles, there 'dumping' the particles into the exit-currents. Particle selection appears to be by size only. Mucus plays no role in feeding in the sense of entrapping particles in mucous film or webs over the faces of the tentacles. The function of the latero-frontal cilia is not known. Microvillous developments of the surface membrane of tentacle epidermal cells have been found and described. Their significance is not understood.

The previously described (Selys-Longchamps, 1907; Marcus, 1949; Silèn, 1952; Lönöy, 1954) acidophil and basiphil epidermal glands have been examined histologically and histochemically. The existence of two biochemically different gland cells has been confirmed, and their secretions

found to be a muco- or glyco-protein and a chondroitin sulfate type B respectively. The function of the proteinaceous gland cell, found in the tentacle and trunk epidermis, is not known. The basiphil gland cells secrete a mucoid film over the trunk of the animal. This same unicellular gland is found in the buccal groove and throughout the digestive tract epithelium.

A study of the main regions of the digestive tract, namely the proventriculus, stomach, and intestine, has shown these regions to have characteristic histochemical and ultrastructural properties. High esterase and phosphatase activity has been demonstrated for the proventriculus and stomach, while no such activity was shown present in the intestinal epithelium. Large numbers of electron-dense granules have been observed in the apical cytoplasm of the proventricular and stomach epithelial cells. Their size, density and location appears consistent with zymogen granules described elsewhere (Palade, 1959; Fawcett, 1966). Lipid droplets, enveloped in 'films' of phosphatase and esterase,* are scattered throughout the epithelium of the proventriculus, suggesting that the descending gut plays a role in lipid absorption and subsequent metabolism. The stomach appears to be the main site of zymogen secretory activity, as evidenced by the profusion of rough endoplasmic reticulum, secretory granules, and other indirect evidence. The intestine appears to act primarily in nutrient absorption via the microvilli lining the epithelial surface and the absorptive vesicles scattered throughout the apical cytoplasm of the cells in this region. The main digestive regions and their morphological character are shown schematically in FIGURE 7.

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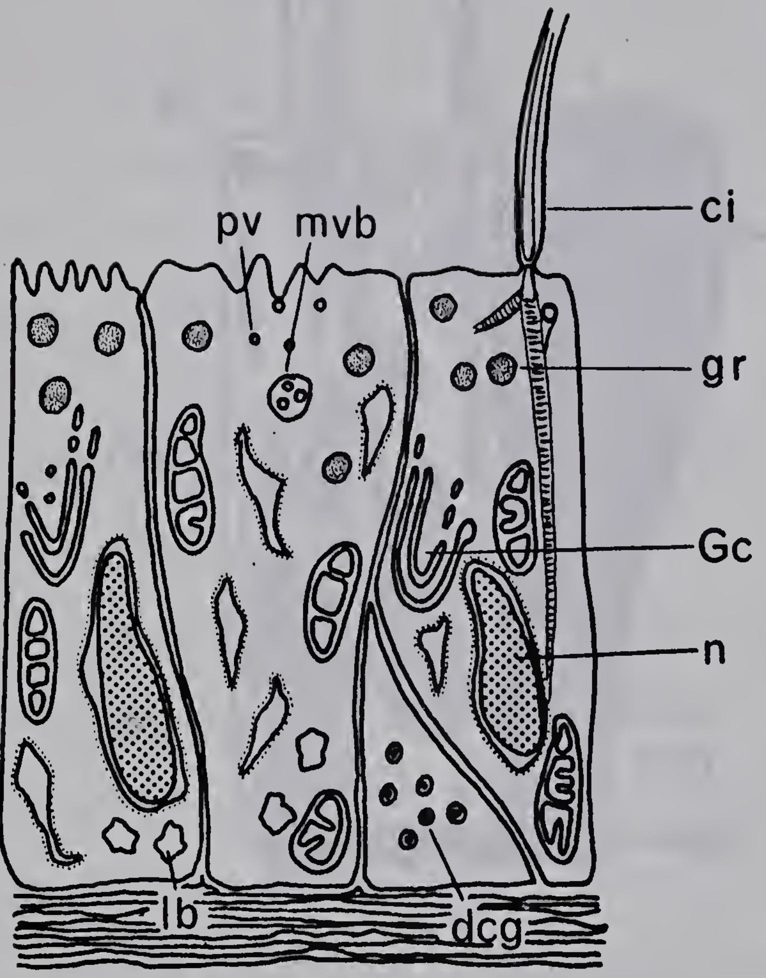
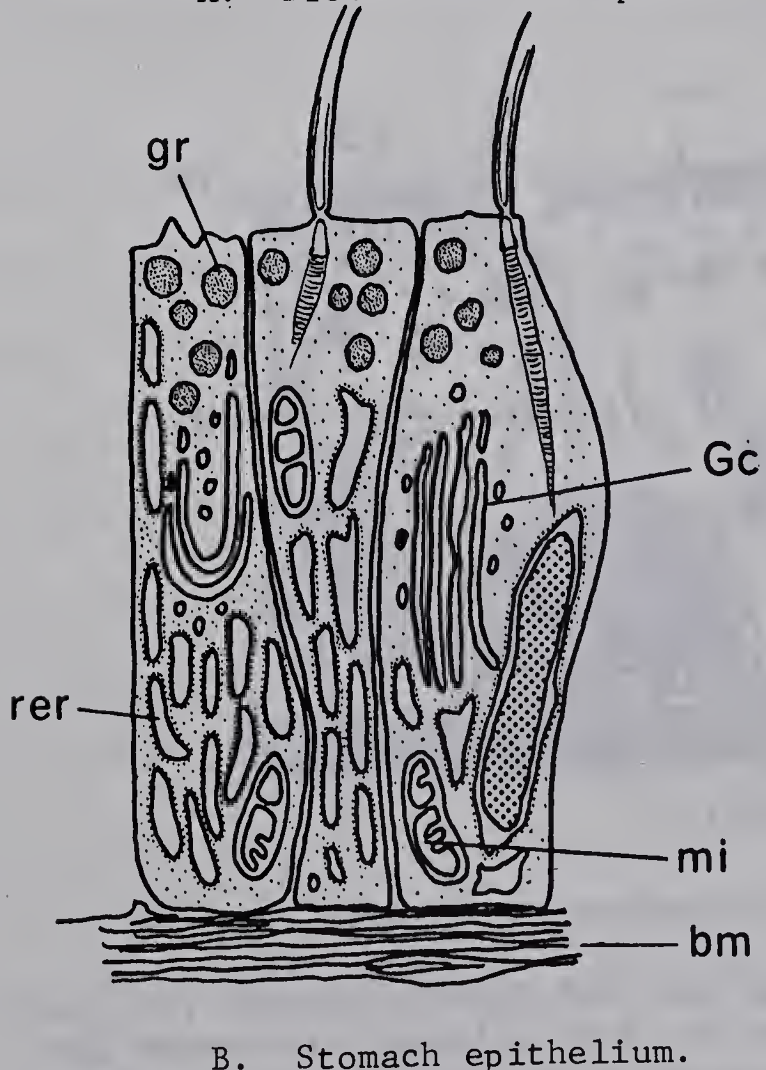
*See footnote page 54.



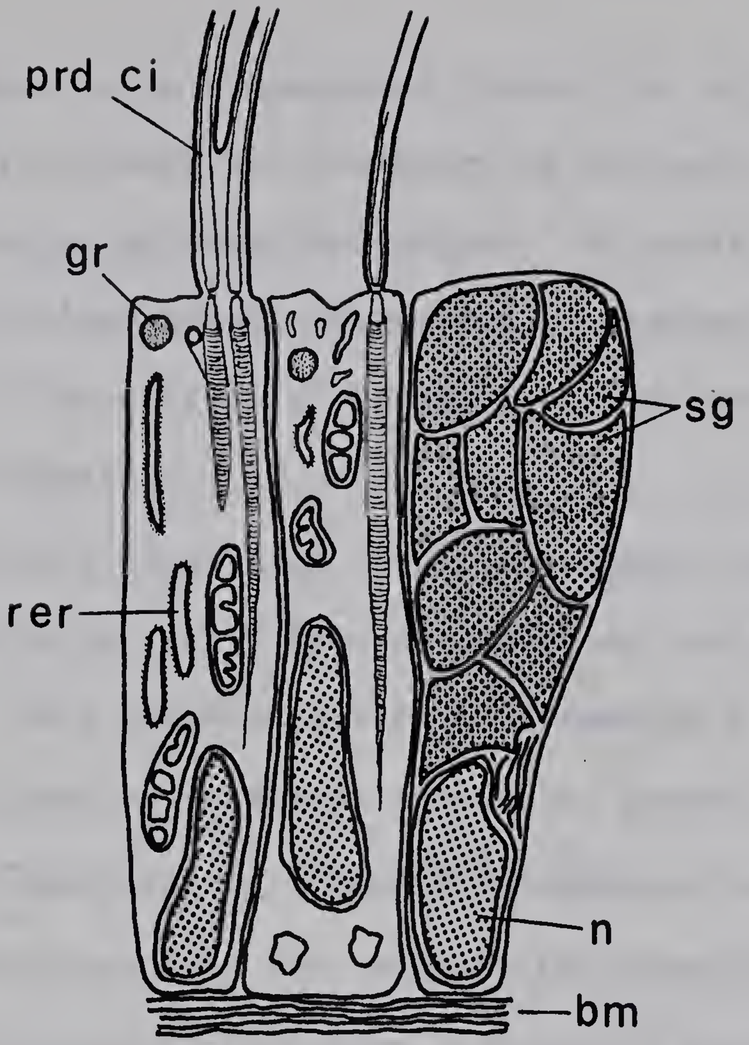
A. Proventricular epithelium.



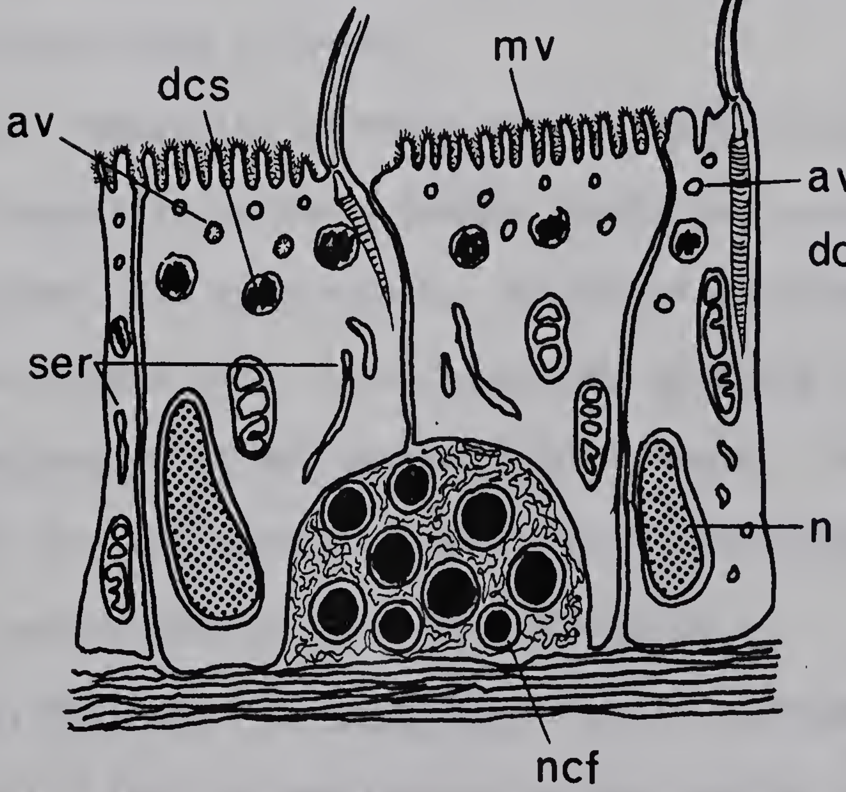
B. Stomach epithelium.

FIGURE 7. Diagramatic representations of epithelial cell types of digestive tract of Phoronis vancouverensis Pixell. For key to abbreviations see p. 64.

[illegible]



C. Intestine - anterior.

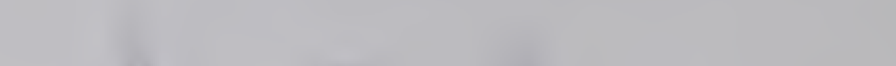


D. Intestine - posterior.

FIGURE 7. Key: av, absorptive vesicle. bm, basement membrane. ci, cilium. dcg, dense-core granule. dcs, dense-core structure. Gc, Golgi complex. gr, electron-dense granule. lb, lipid body. mi, mitochondrion. mv, microvillus. mvb, multi-vesicular body. n, nucleus. ncf, non-cellular figure. prd ci, paired cilia. pv, 'pinocytotic' vesicle. rer, rough endoplasmic reticulum. ser,smooth endoplasmic reticulum. sg, secretory globule.

SC

[illegible]



[illegible]

[illegible]

Particles appear to be transported through the digestive tract by the movement of cilia lining the periphery of the gut. No ciliated ridges or food gutters per se were discernible. In addition, no evidence was found of holophagocytic digestion of diatoms, or of storage of diatom skeletons in the epithelial walls of the stomach and the anterior part of the intestine.

A few cells with a pronounced 'dark' appearance, when observed with the electron microscope, have been described in the intestine of Ph. vancouverensis. Their occurrence has been compared with similar 'dark' cells in the digestive epithelia of certain mammals. Small granules, similar in appearance to argentaffin granules or neurosecretory granules, have been observed in a few cells in the digestive tract of this animal. Their function is not known. Masses of degenerate epithelial tissue containing large, intensely electron-dense, spherical structures have been observed in the basal regions of the intestinal epithelium. Neither their function nor fate is known.

Electrophoretic separation of whole animal extract has demonstrated a full complement of digestive enzymes, namely, amylases, esterases, acid and alkaline phosphatases, and gelatinases. Of the seven esterases found, three appear to be associated with lipid digestion, as shown by their lability following extraction of gut extract with butanol. The gelatinases also can be separated into two groups on the basis of different pH optima and their respective sensitivity to cysteine activation.

In conclusion, Phoronis vancouverensis Pixell, although apparently totally dependent for its food on particulate matter carried into the lophophore in the incoming water currents, compensates by moving large amounts of water through the lophophore (up to 2.5 l/hr). The organism

does not appear to have a mechanism for selecting particles qualitatively. Rather, it sorts particulate matter mechanically, on the basis of size only. Digestion in this animal appears to occur in part extracellularly in the proventriculus and stomach, by secretion of zymogen granules into the lumen of the gut. The presence of microvilli and absorptive vesicles suggests that subsequent absorption of the resulting products of enzyme hydrolysis occurs distally in the intestine.

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VII. EXPLANATION OF PLATES

Plates 12, 15, 16, and 43 - 46 are light micrographs; the remaining plates are all electron micrographs. In all cases originals were made, labelled and reproduced photographically.

For the electron micrographs the preparation of tissue was identical throughout. Tissue was fixed in S-collidine buffered osmium tetroxide, embedded in Epon, and stained with uranyl acetate - lead citrate (viz. MATERIALS AND METHODS).

PLATE 1. Transverse section of tentacle gland cells. Note three types of nuclei (n), and dilated nuclear envelope (ne).

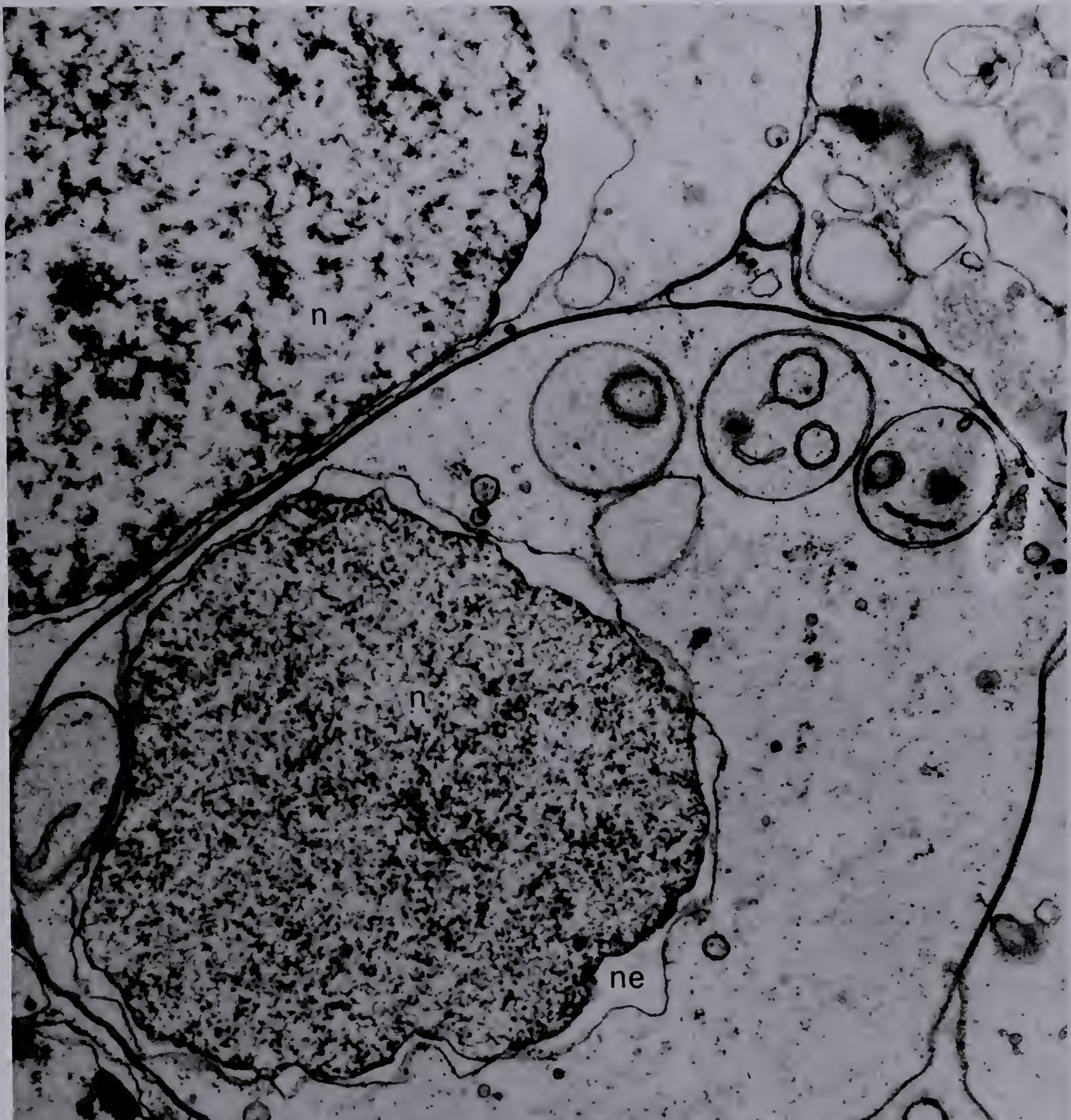

n
n
ne
n
1 μ
1

PLATE 2. Section through tentacle aboral gland cell, showing dilated nuclear envelope (ne), with ribosomes arrayed along outer membrane (arrow). Note elements of distended rough endoplasmic reticulum (rer).

②

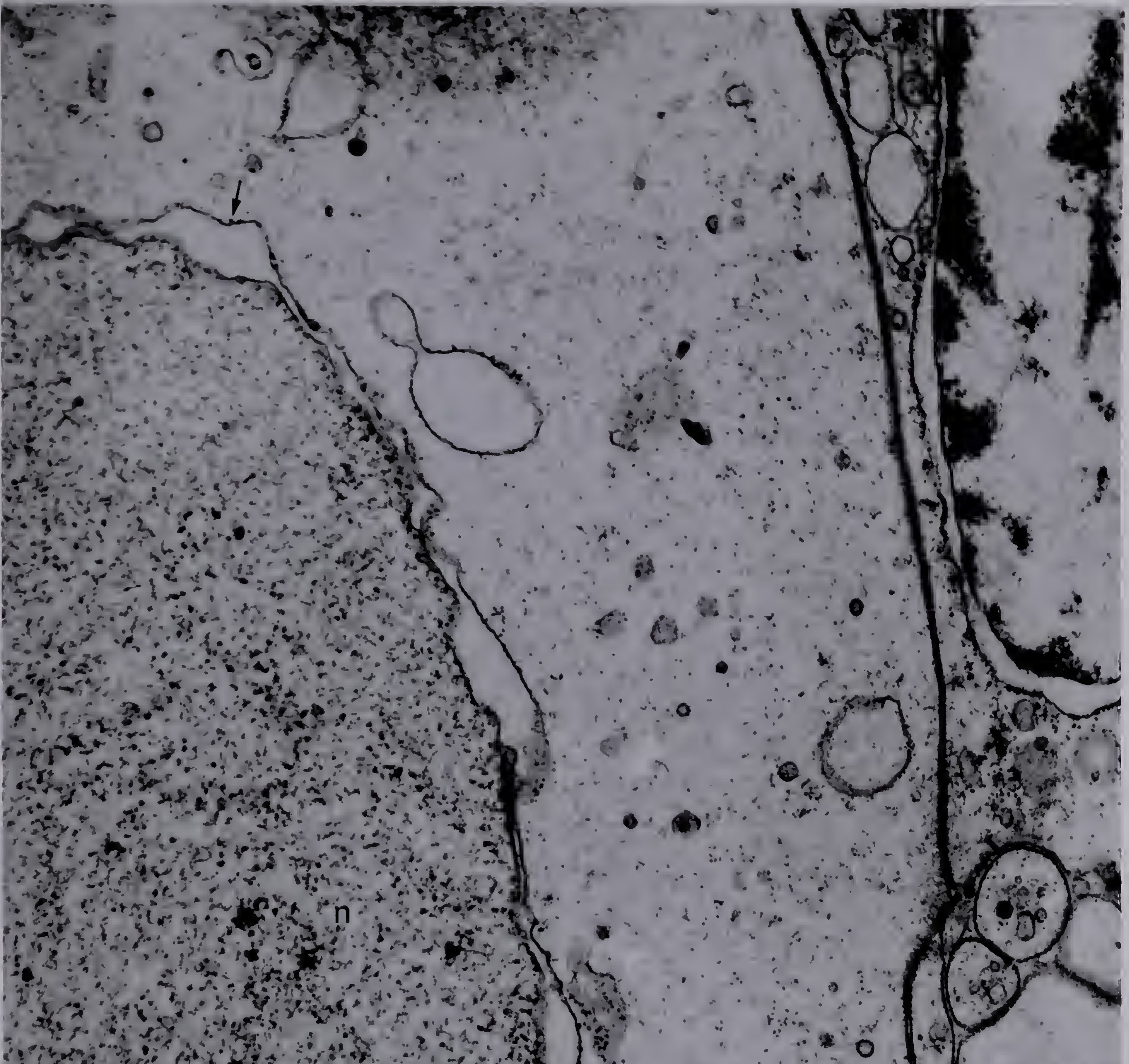


PLATE 3. Apical portion of tentacle gland cell. Gc, Golgi complex. mvb, multi-vesicular body. rer, rough endoplasmic reticulum. scm, surface cell membrane. sg, secretory globule.

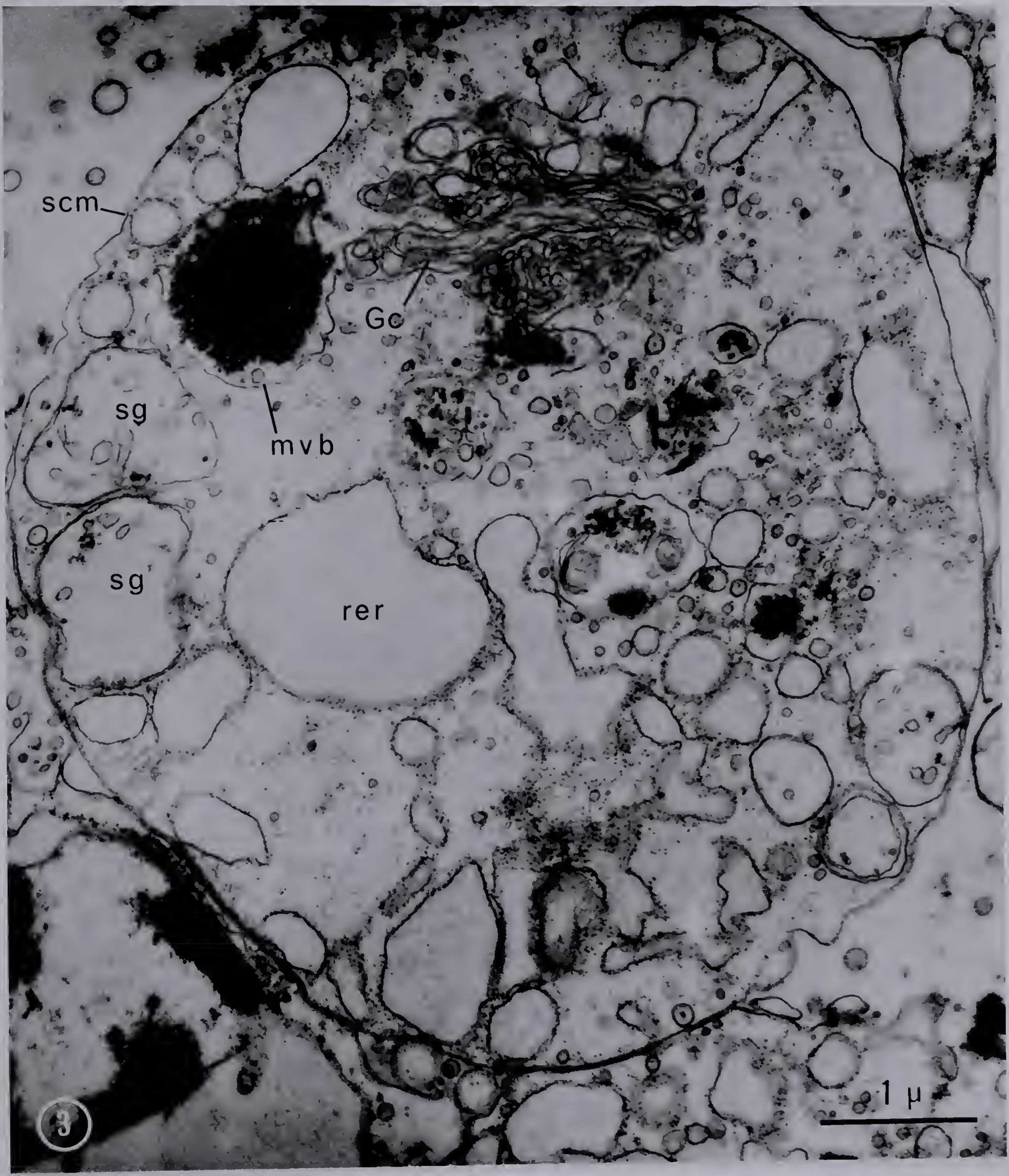

scm
Gc
sg
mvb
sg
rer
1 μ
3

PLATE 4. Apical portion of unspecialized tentacle cell. Note convoluted surface cell membrane (scm), and interleaving of adjacent cells (arrow).

ci, cilium cross-section. n, nucleus. ne, dilated nuclear envelope.

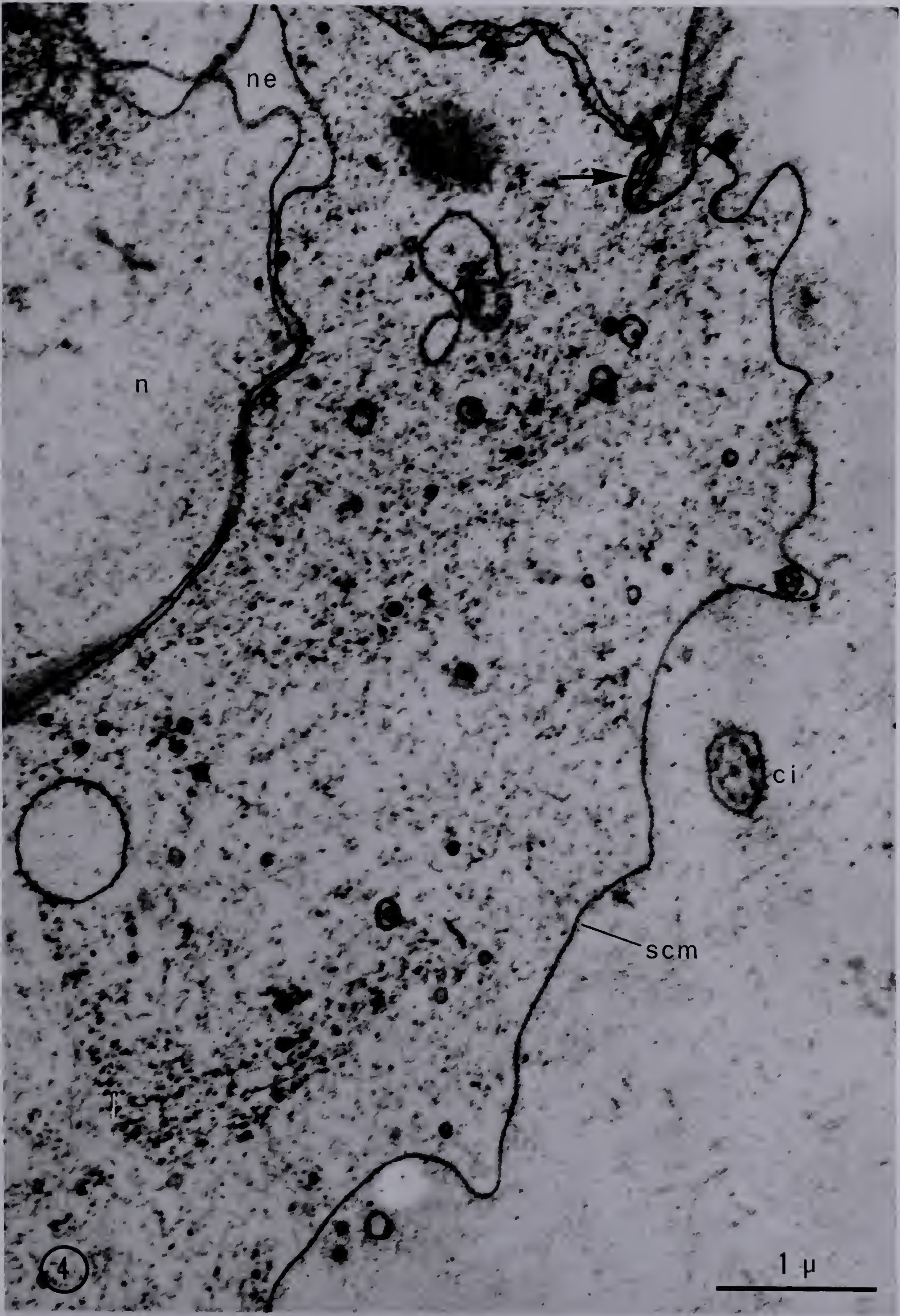

ne
n
ci
scm
l
4
1 μ

PLATE 5. Distal portions of cells from aboral tentacle face showing outbulgings of surface cell membrane (arrows) and microvilli (mv).

s, spherule with coat material.

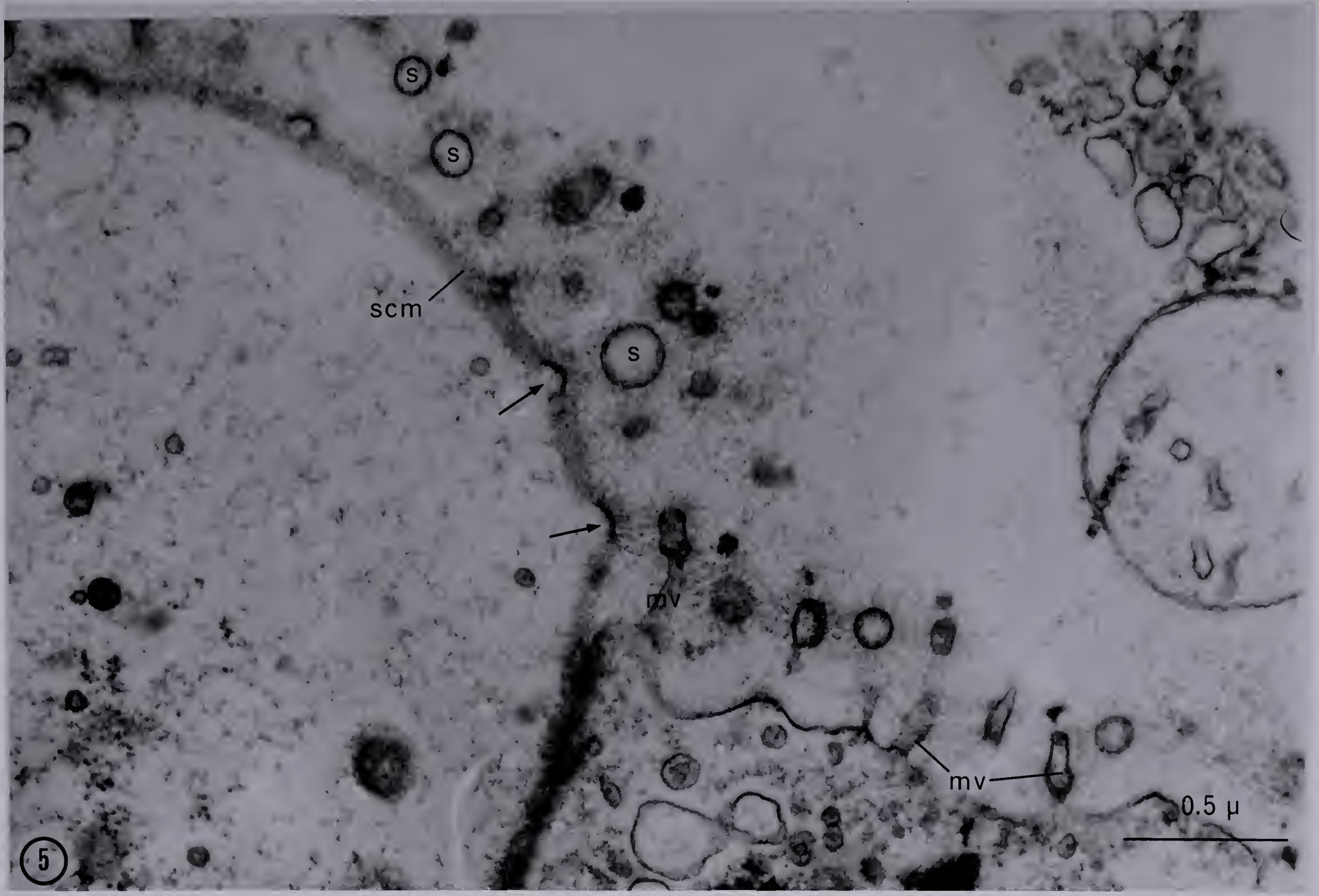
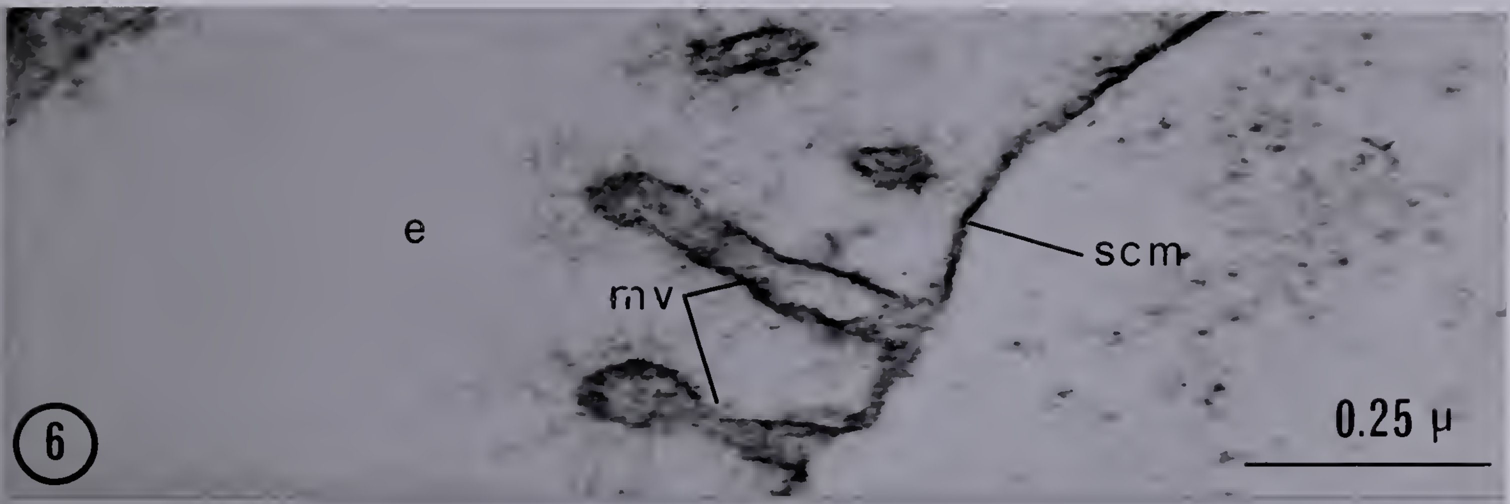
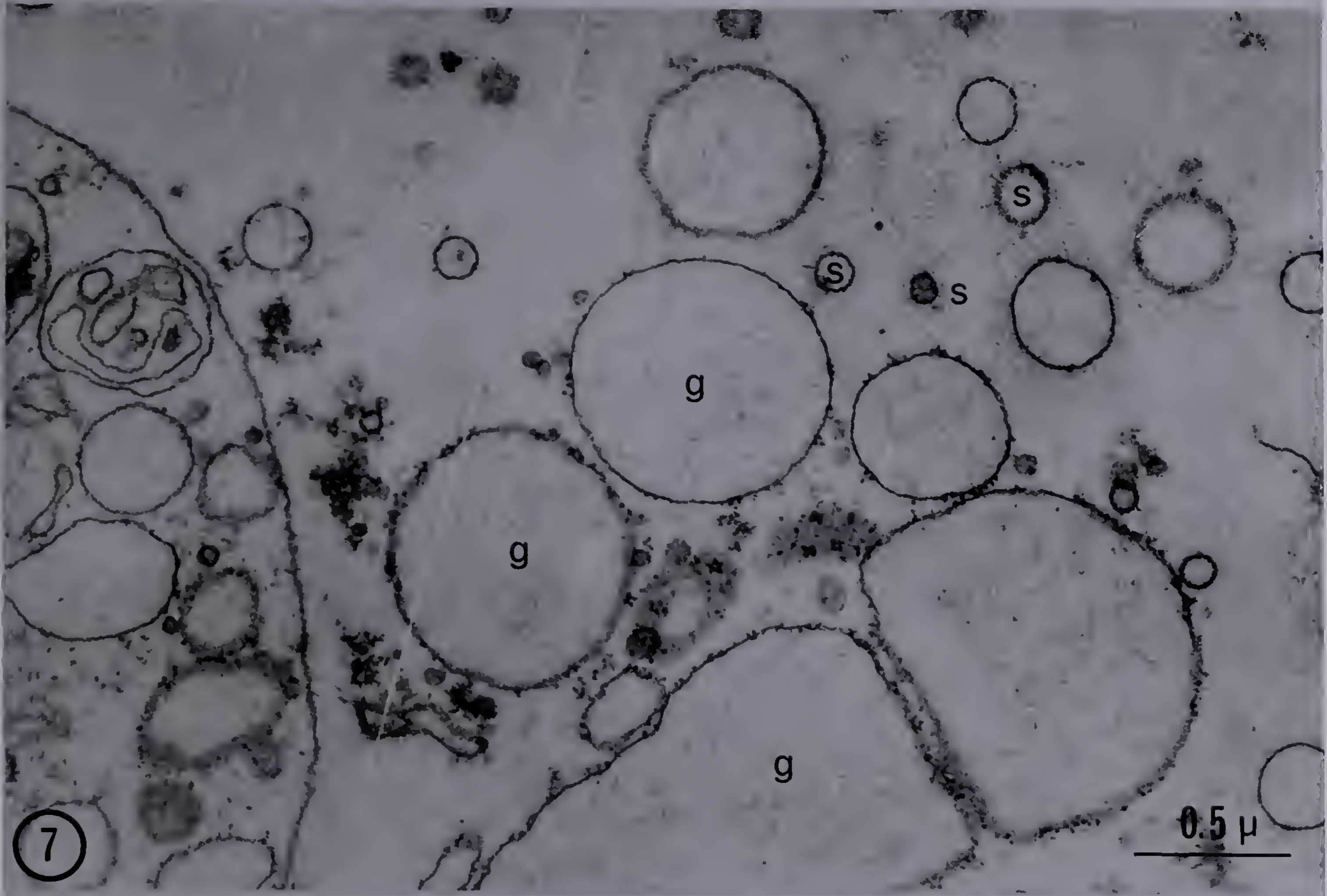

s
s
scm
s
mv
mv
0.5 μ
5

PLATE 6. Microvilli extending into extratentacular space. e, extracellular space. mv, microvillus. scm, surface cell membrane.

PLATE 7. Globular secretory matter (g) lying in extratentacular space. Note lack of coat material. Scattered throughout are smaller spherules (s) covered with coat material.

PLATE 8. Spherules (s) covered with coat material (arrow), and secretory globules (g) lying in the extratentacular space.


e
mv
scm
0.25 μ
⑥


s
s
s
g
g
g
0.5 μ
⑦

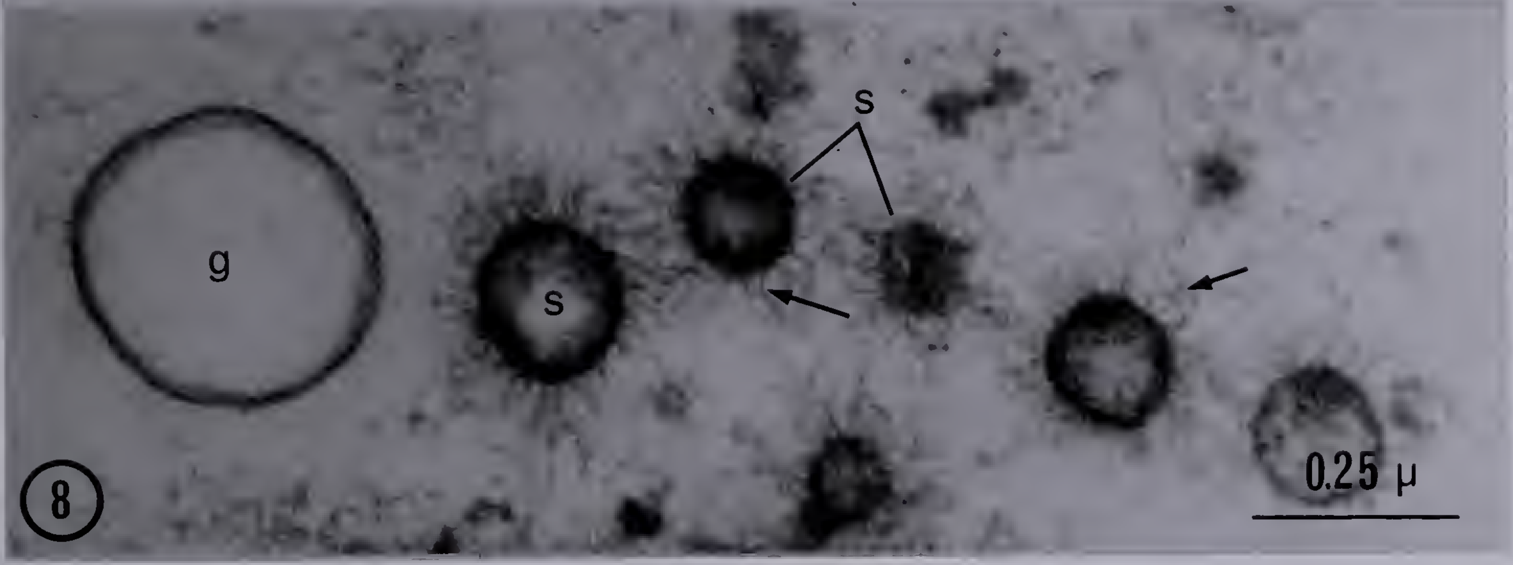
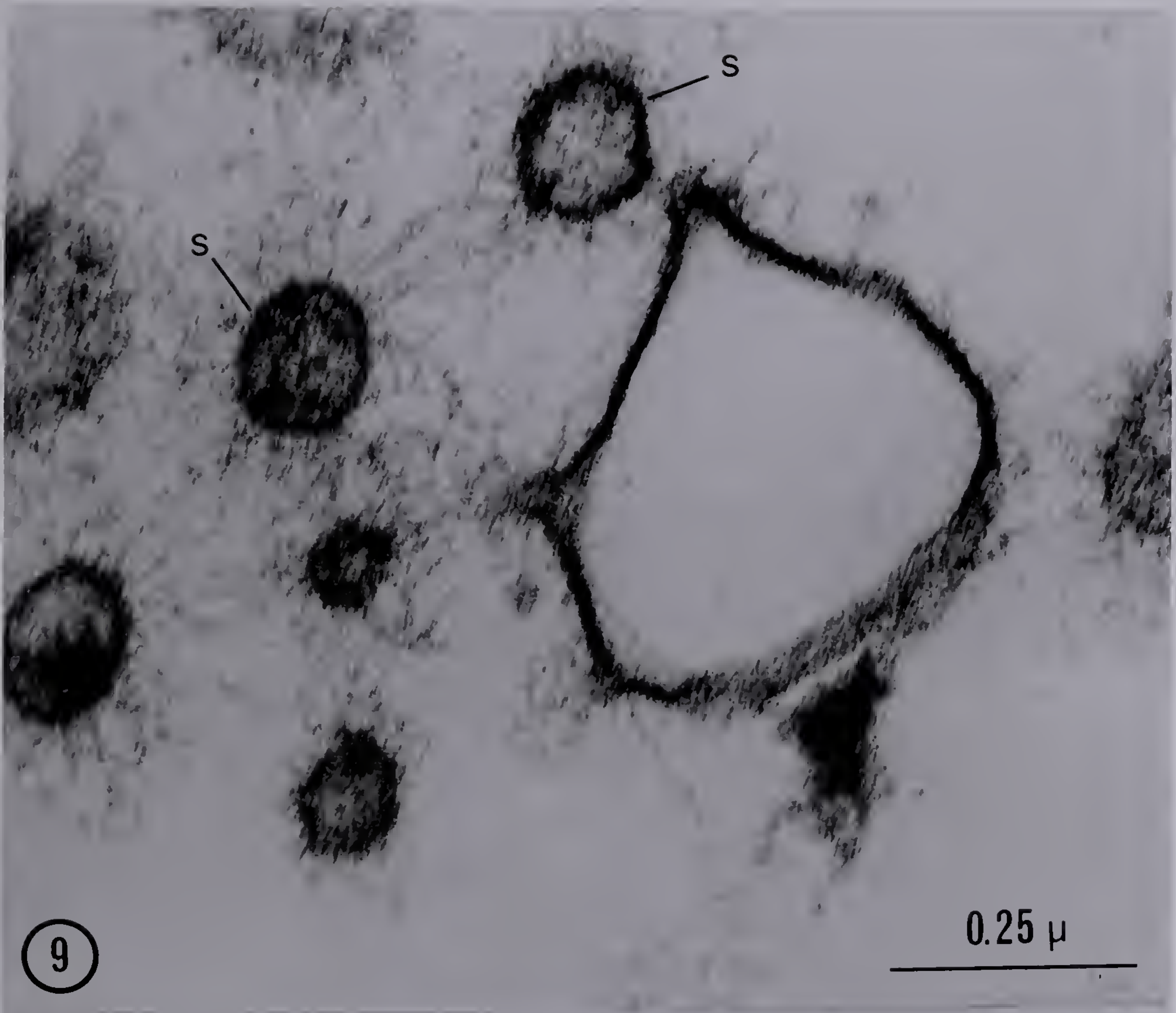

s
g
s
0.25 μ
⑧

PLATE 9. Spherules (s) with coat material. Note larger irregularly shaped structure.

PLATE 10. High magnification electron micrograph of spherule, showing double character of limiting membrane (arrows). Note fine filaments of coat material (f) radiating outward from limiting membrane.


s
s
9
0.25 μ

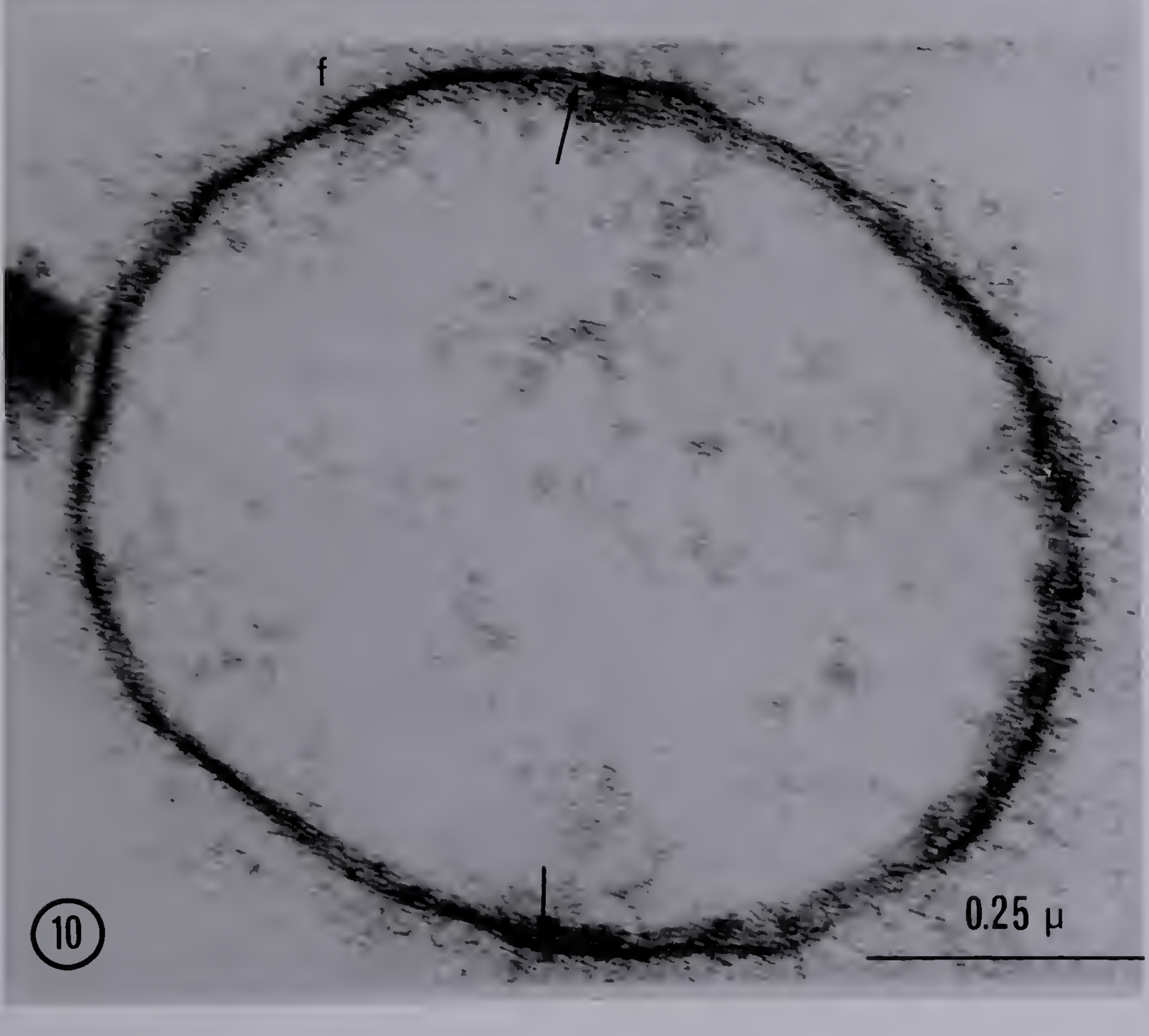

f
10
0.25 μ

PLATE 11. Transverse section of trunk epidermis, showing two unicellular gland types.

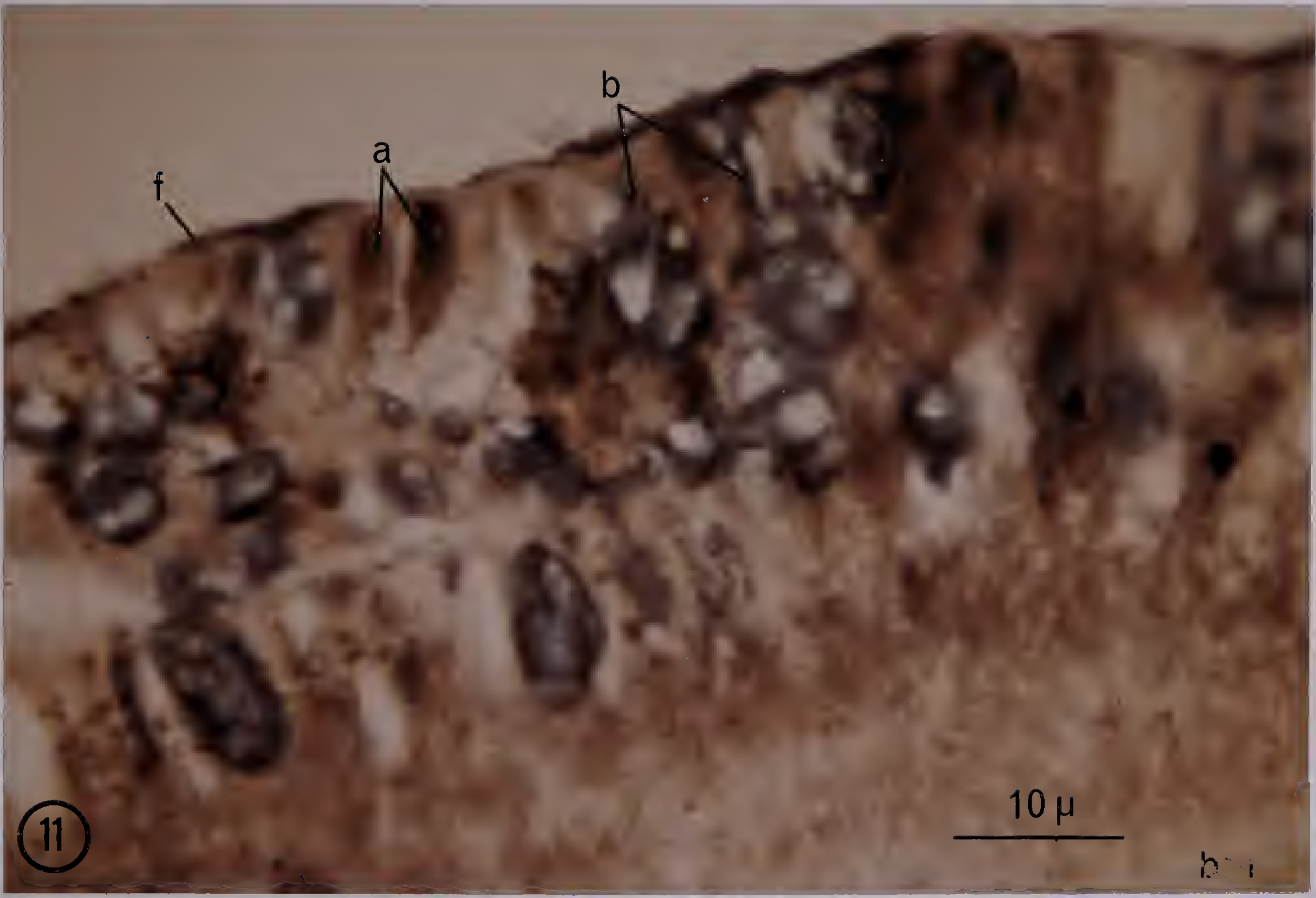
a, acidophil gland cell. b, basiphil gland cell.

bm, basement membrane. f, film secreted over trunk epidermis.

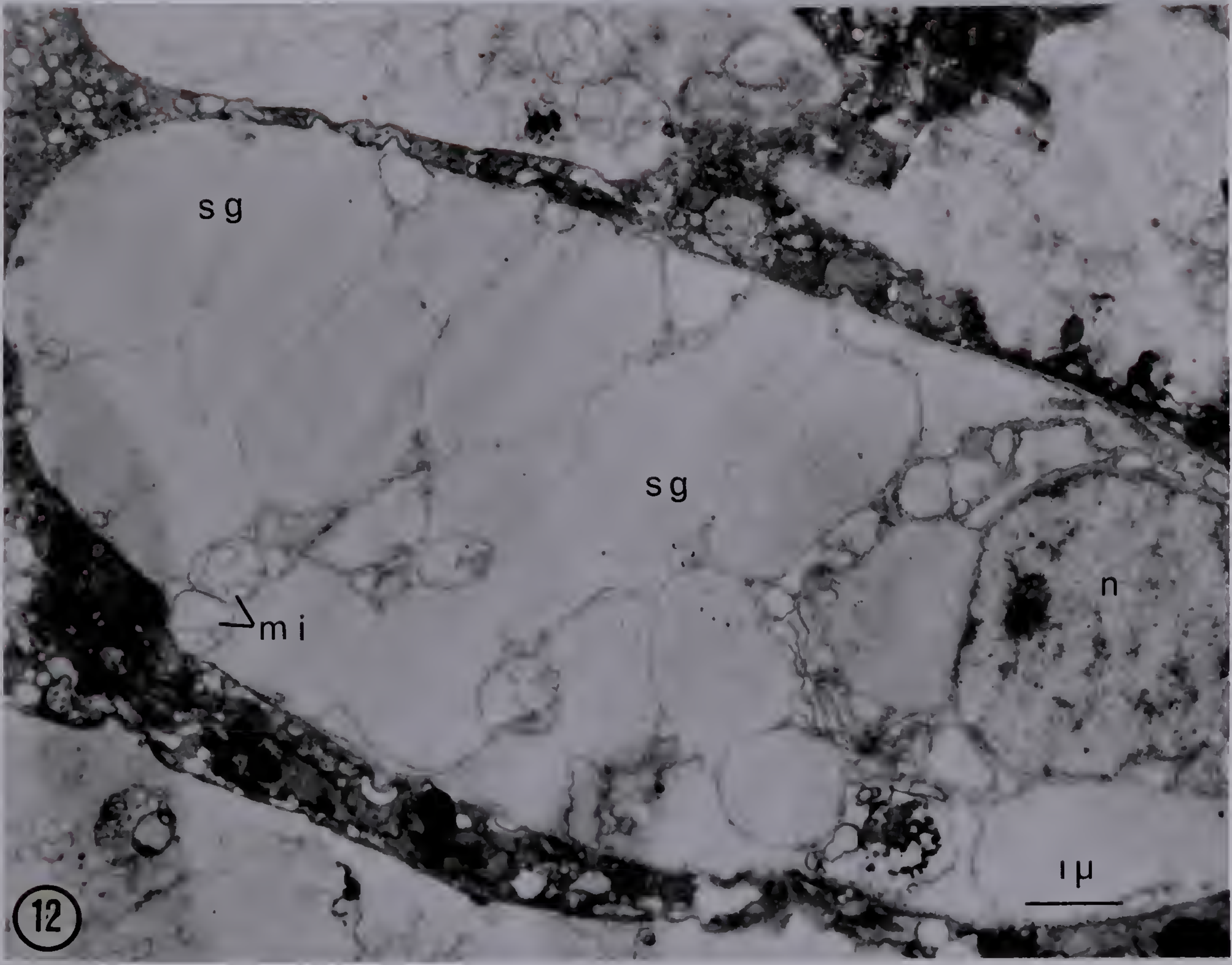
Susa fixation, paraffin embedding, Alcian Blue-tetrazotized Benzidine.

PLATE 12. Section through apical portion of preserved basiphil gland cell in trunk epidermis. Note compacted mass of secretory globules, and basal location of nucleus.

mi, mitochondrion. n, nucleus. sg, secretory globules.



11

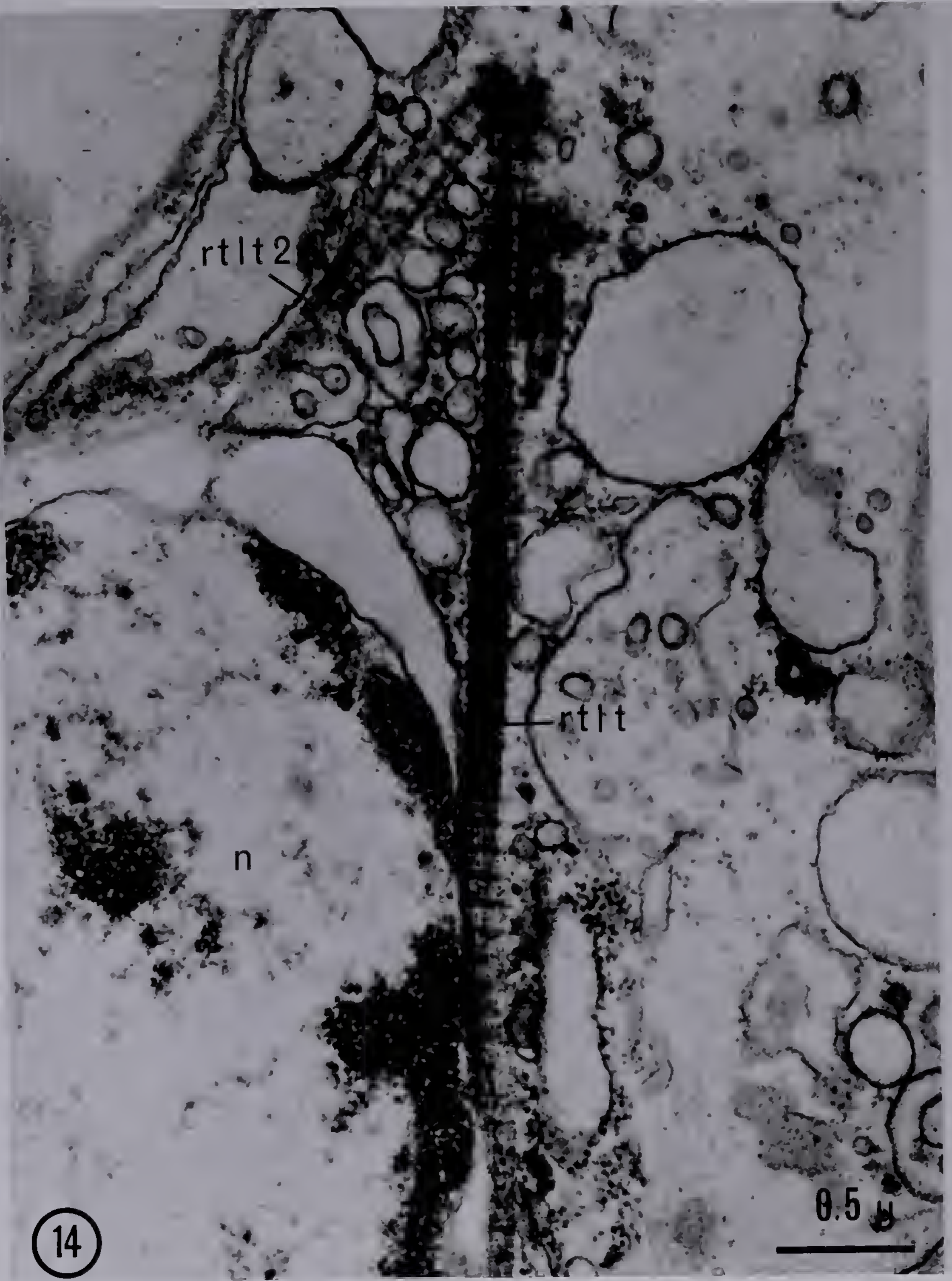


12

PLATE 13. Distal portion of ciliated (ci) tentacle cell. bb, basal body. bp, basal plate.

PLATE 14. Section through apical portion of ciliated tentacle cell, showing rootlet system of ciliary apparatus. Basal bodies and cilium not shown in this section.

n, nucleus with dense chromatin patches. rtlt, rootlet. rtlt2, short second rootlet.


⑭

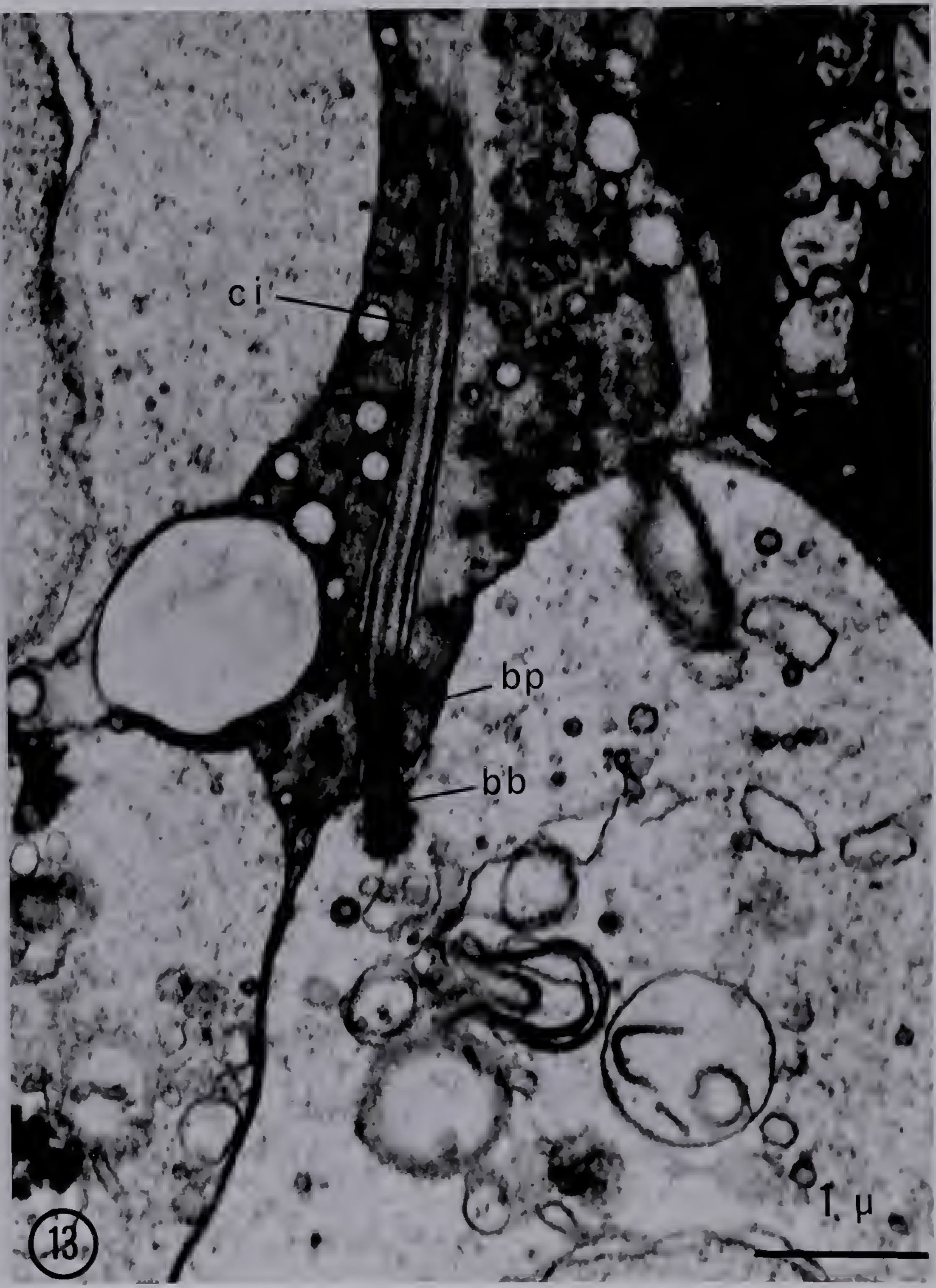

⑬

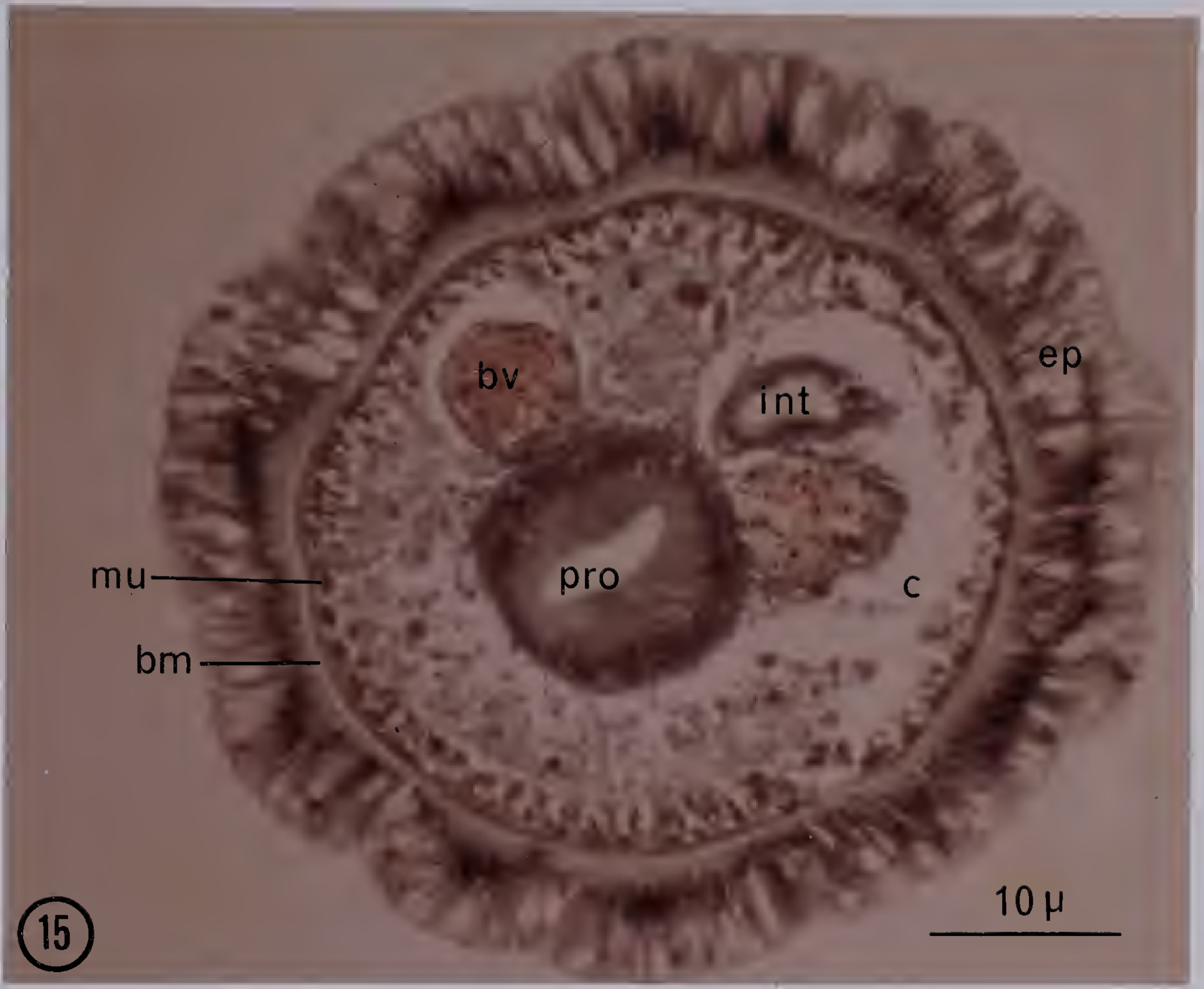
PLATE 15. Cross-section through mid-trunk region of *Phoronis vancouverensis* Pixell.

bm, basement membrane. bv, blood vessel. c, coelom. ep, epidermis. int, intestine. mu, muscle. pro, proventriculus.

Susa fixation, paraffin embedding, Masson trichrome.

PLATE 16. Cross-section through proventricular epithelium. Note small granules in apical cytoplasm (arrows). bm, basement membrane. lu, lumen of the gut. Susa fixation, paraffin embedding, periodic acid-Schiff.

15



16

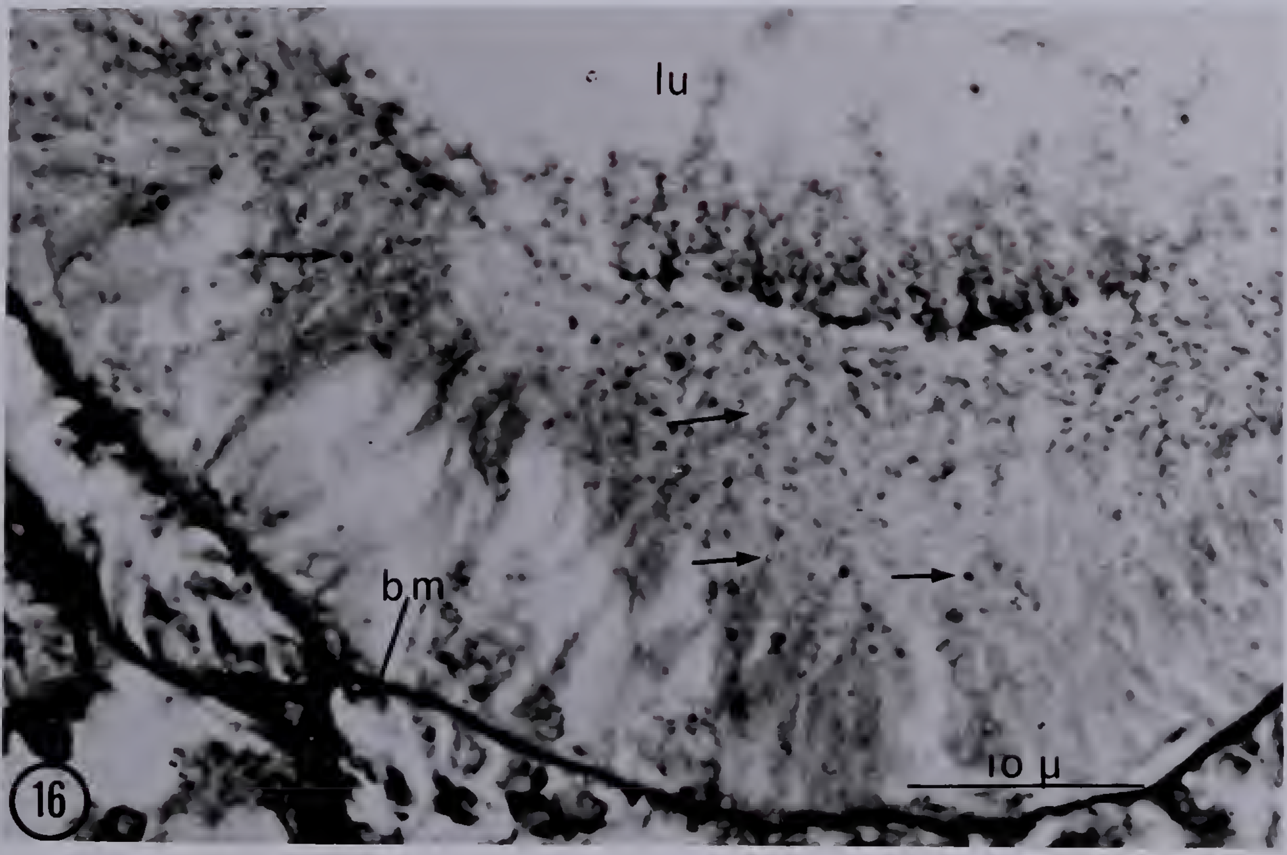


PLATE 17. Cross-section through proventricular epithelial cells. Note large number of electron dense granules (gr).

lf, lamellate figure. mvb, multi-vesicular body. n, nucleus. rer, rough endoplasmic reticulum.

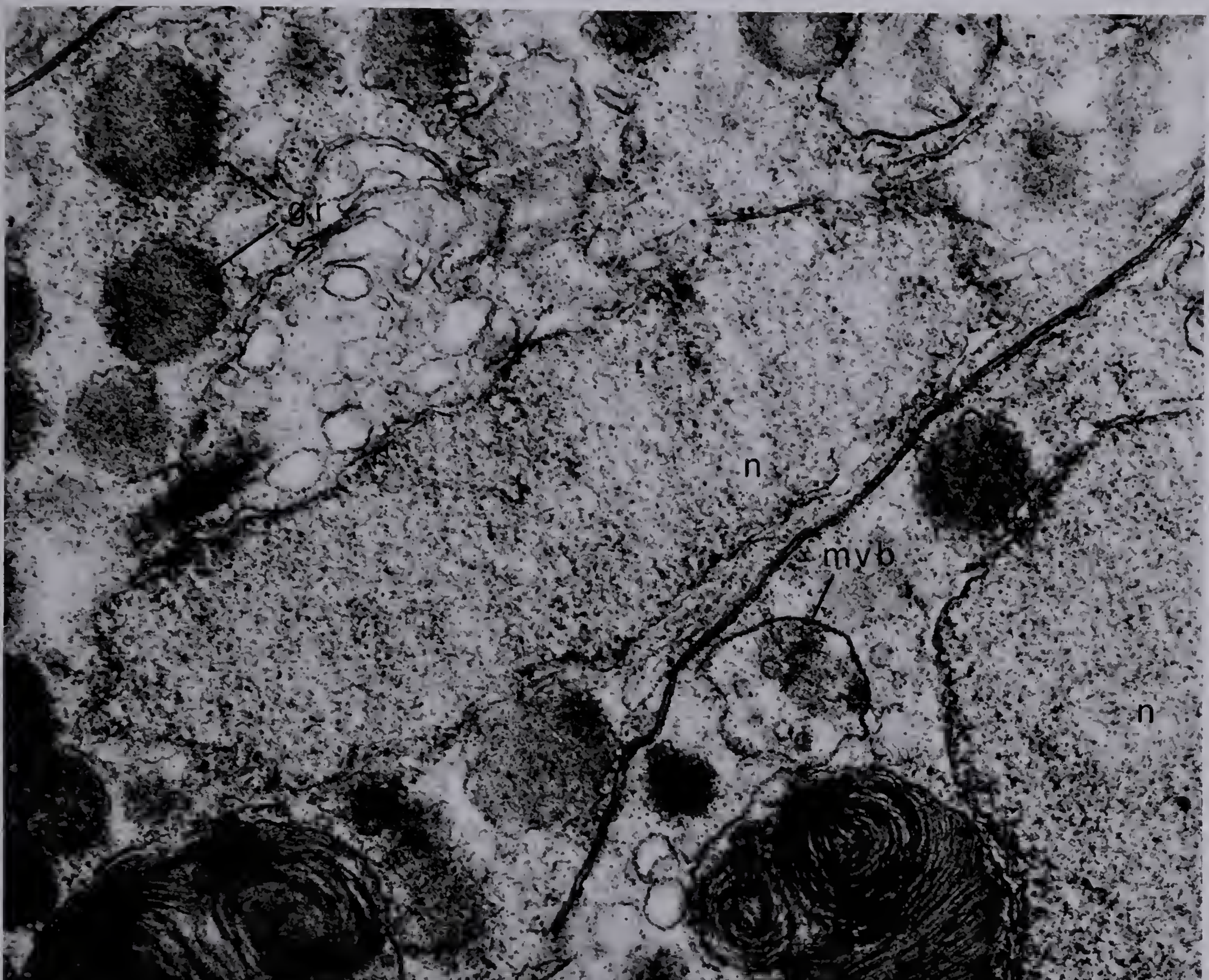

gr
n
mvb
n
lf
gr
rer
17
0.5 μ

PLATE 18. Section through apical portion of a proventricular epithelial cell. The surface cell membrane (scm) exhibits several infoldings. Note apparently pinocytotic vesicles (pv) in process of budding off into intra-cellular matrix.
er, endoplasmic reticulum. gr, electron dense granule. mi, mitochondrion. n, nucleus.

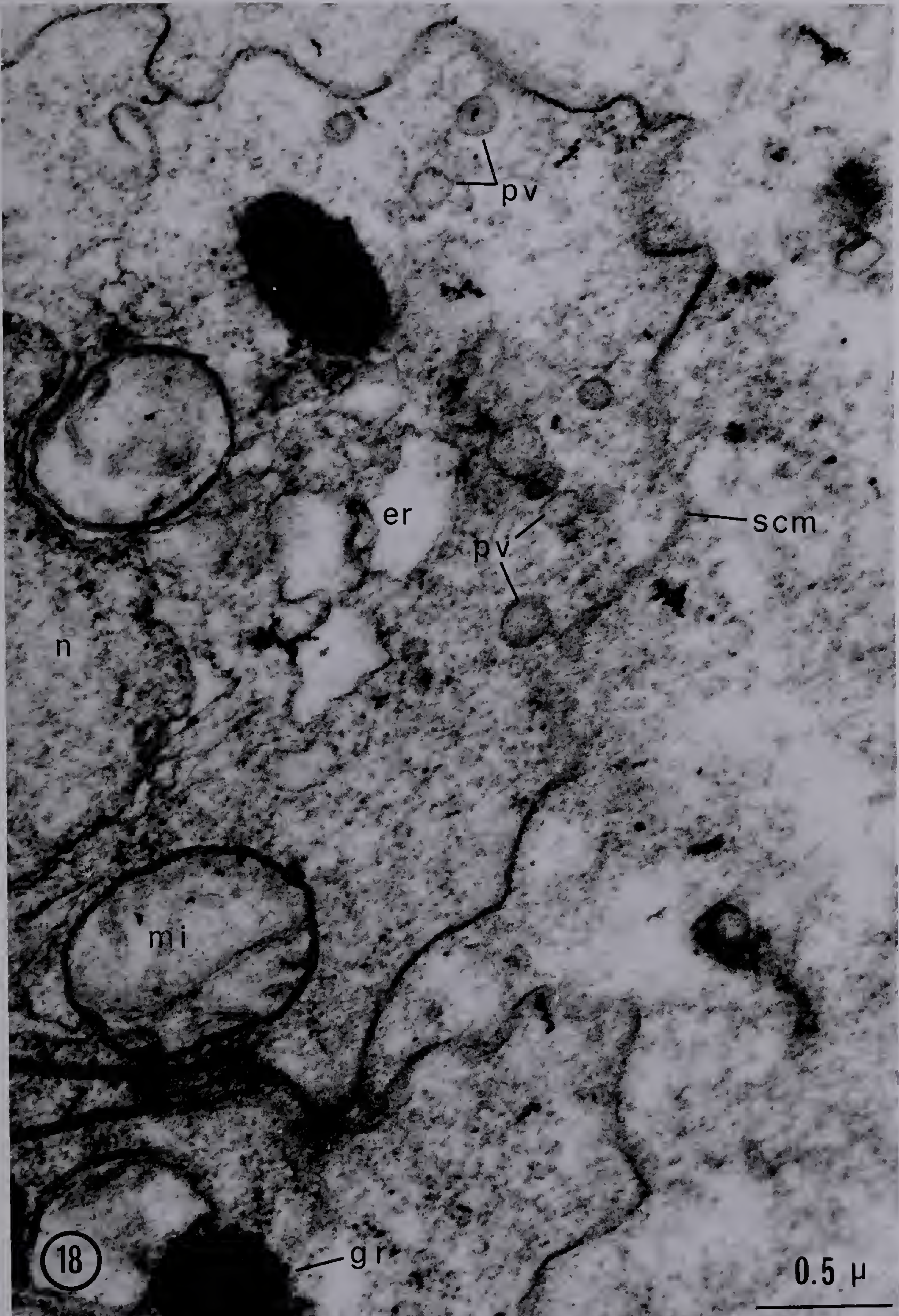

pv
er
pv
scm
n
mi
18
gr
0.5 μ

PLATE 19. Section through proventricular epithelial cell, showing Golgi complex (Gc), and in near proximity several small vesicles (v).

lf, lamellate figure. mvb, multivesicular body.

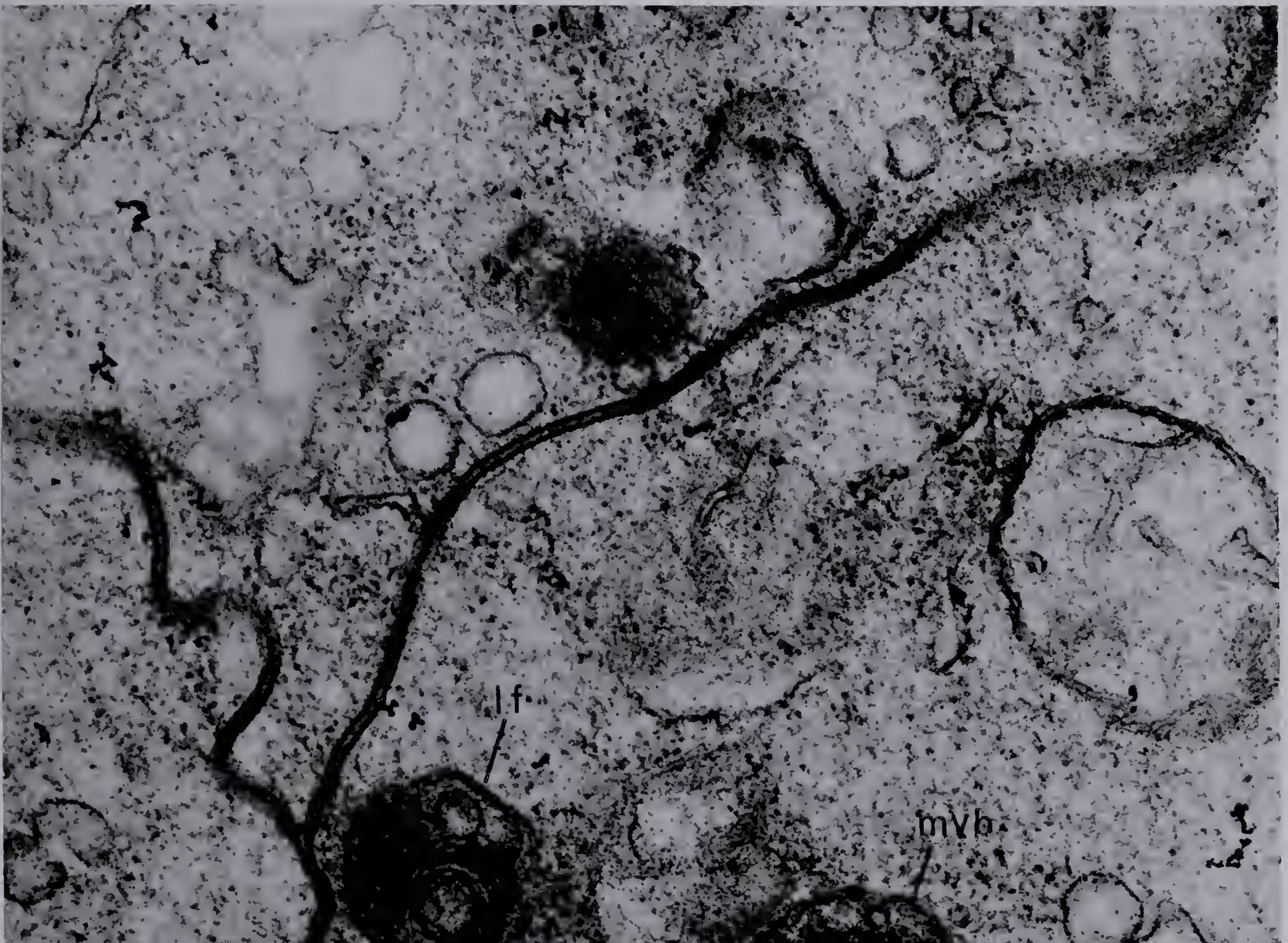

lf
mVb
Gc
v
0.5 μ
⑲

PLATE 20. Golgi complex. The Golgi elements lie equidistant and parallel to one another. Within the innermost concave element lie several small vesicles (v). Note the intimate association of many vesicles and elements of endoplasmic reticulum (er). The tips of Golgi cisternae are swollen into distended saccules, apparently in process of pinching off from the main complex (arrows).

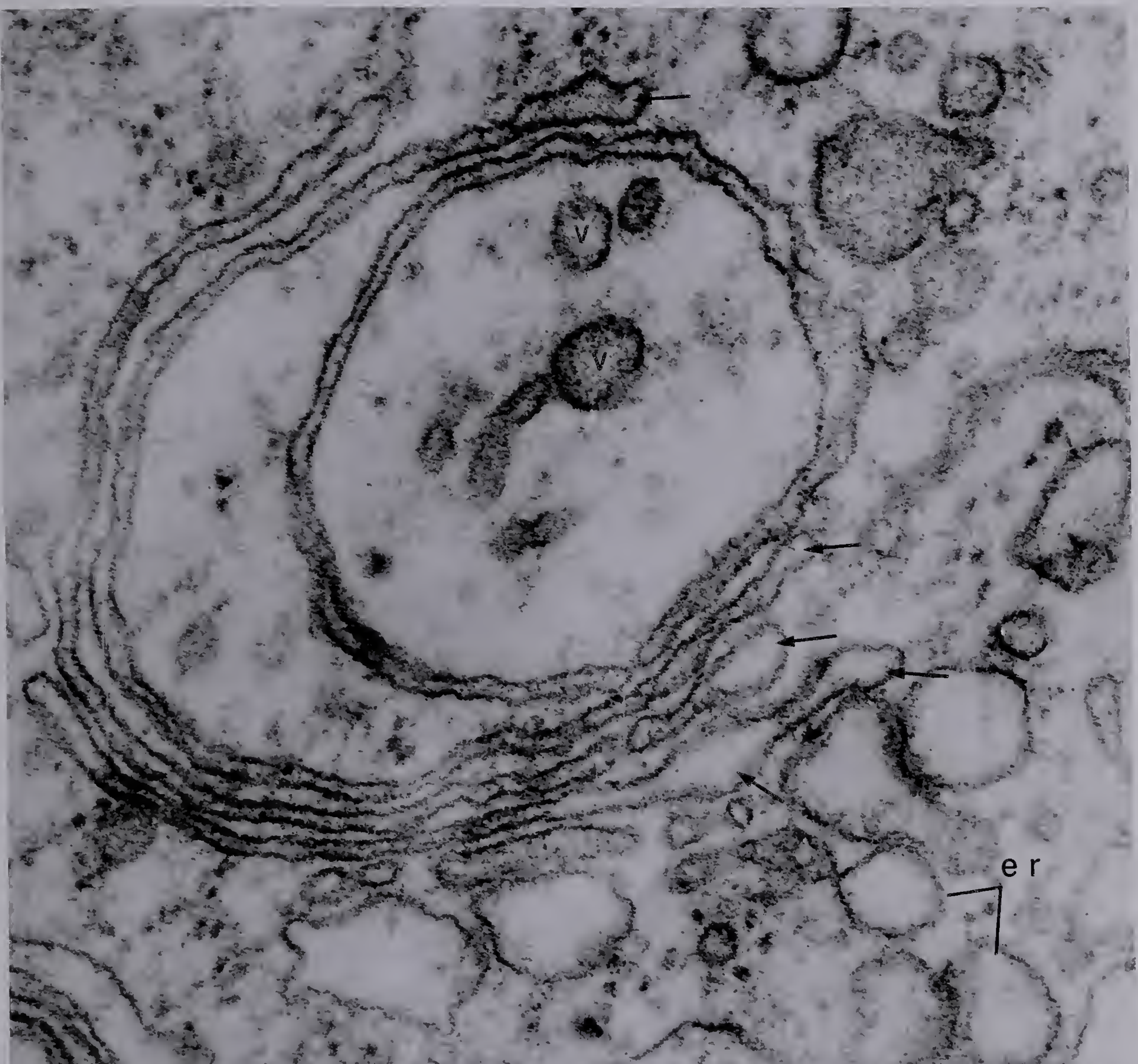

v
v
e r
20
0.25 μ

PLATE 21. Portion of a proventricular epithelial cell containing a ciliary rootlet (rtlt).
bb, basal body. gr, electron-dense granule.

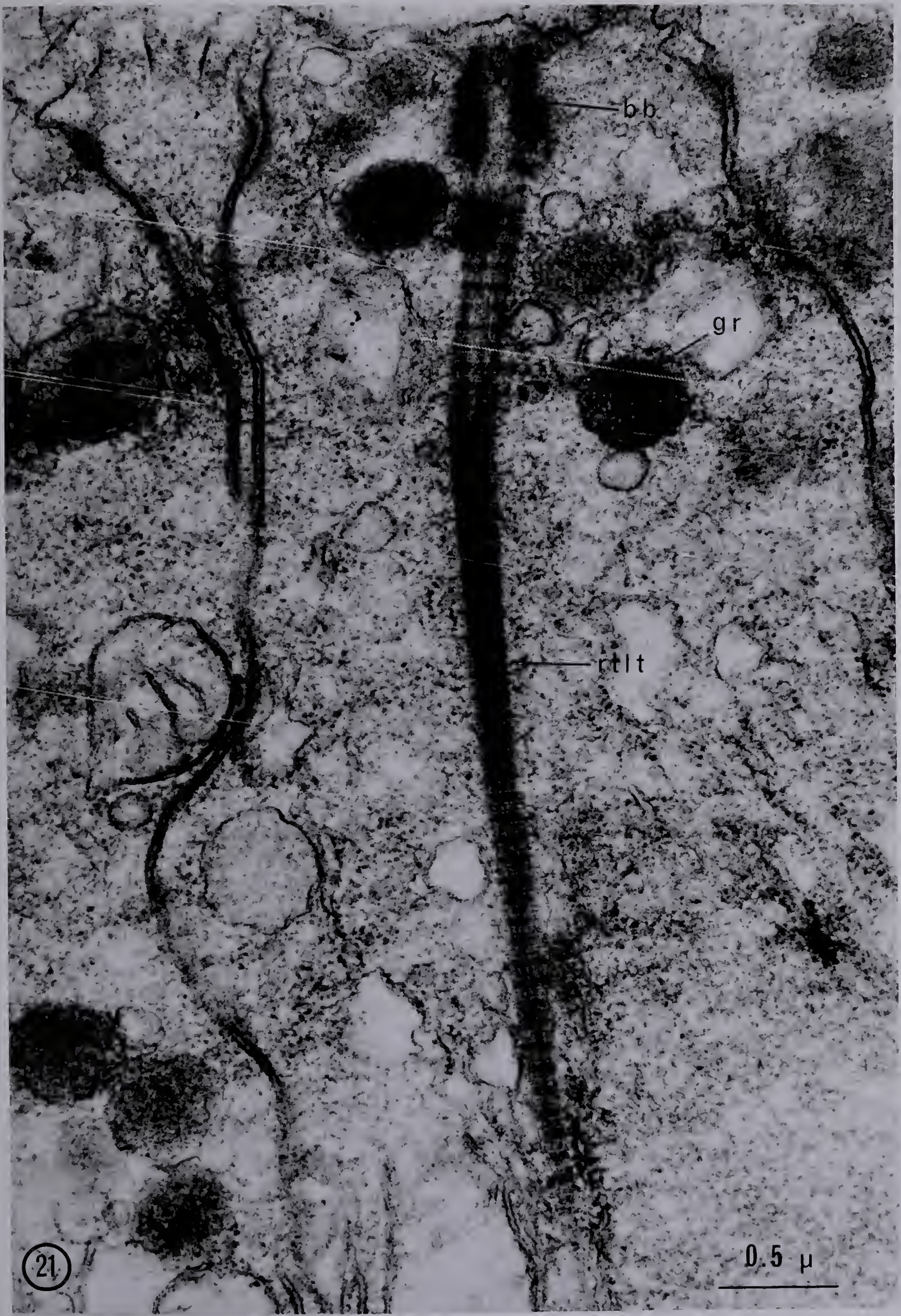

bb
gr
rtlt
0.5 μ
21

PLATE 22. Ciliary apparatus in proventricular region of digestive tract. The cilium (ci) terminates in a basal plate (bp), which is separated from the basal body (bb) by a brief clear space (arrow). Note a second basal body (bb2) lying at right angles to the first basal body.

rtlt, rootlet. rtlt2, short second rootlet.

scm, surface cell membrane.

22

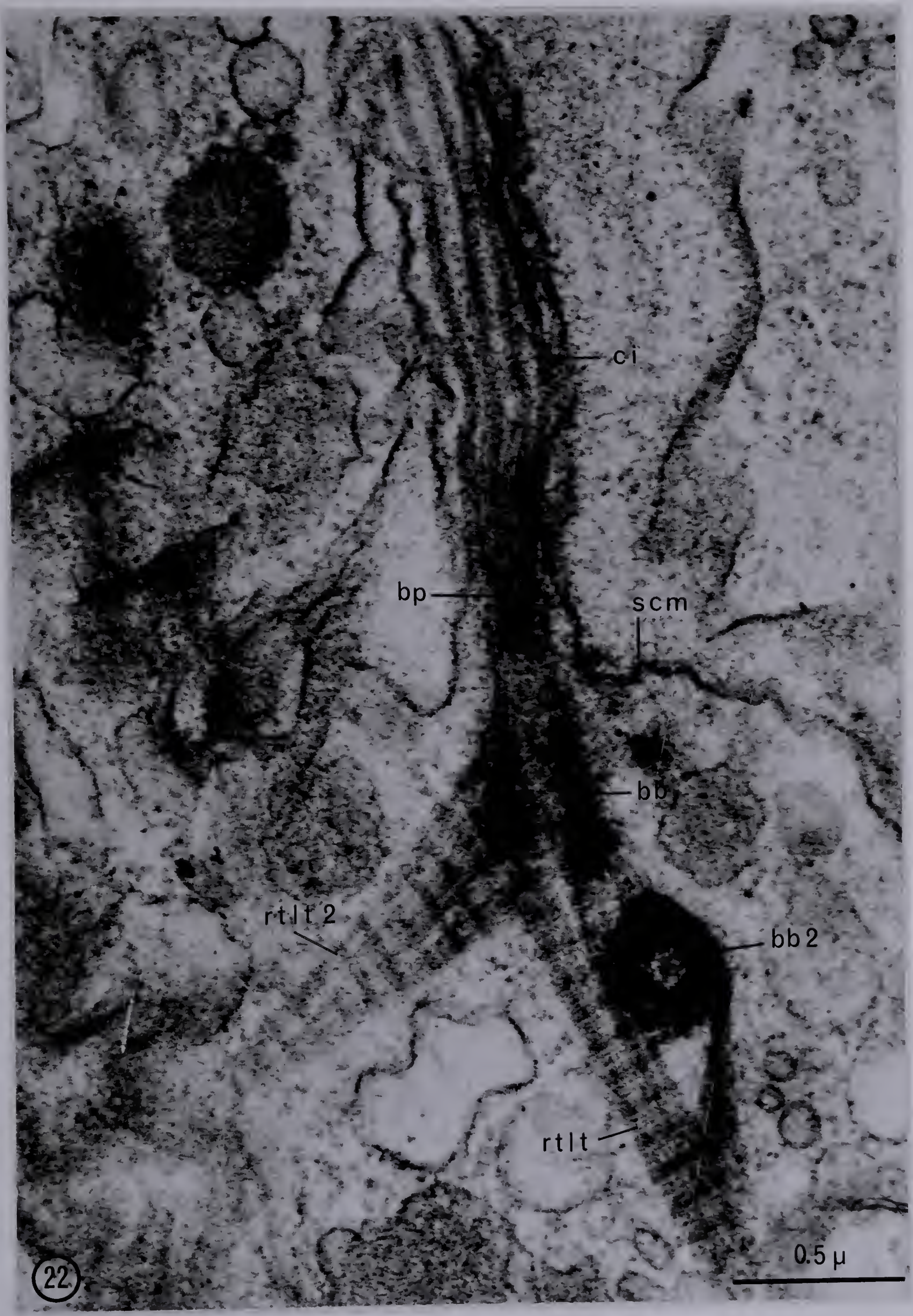


PLATE 23. Section through basal portion of a proventricular epithelial cell. Lipid bodies (lb) lie scattered throughout cytoplasm. Note the mitochondrion in intimate association with two of the lipid bodies (mi).

n, nucleus.

23

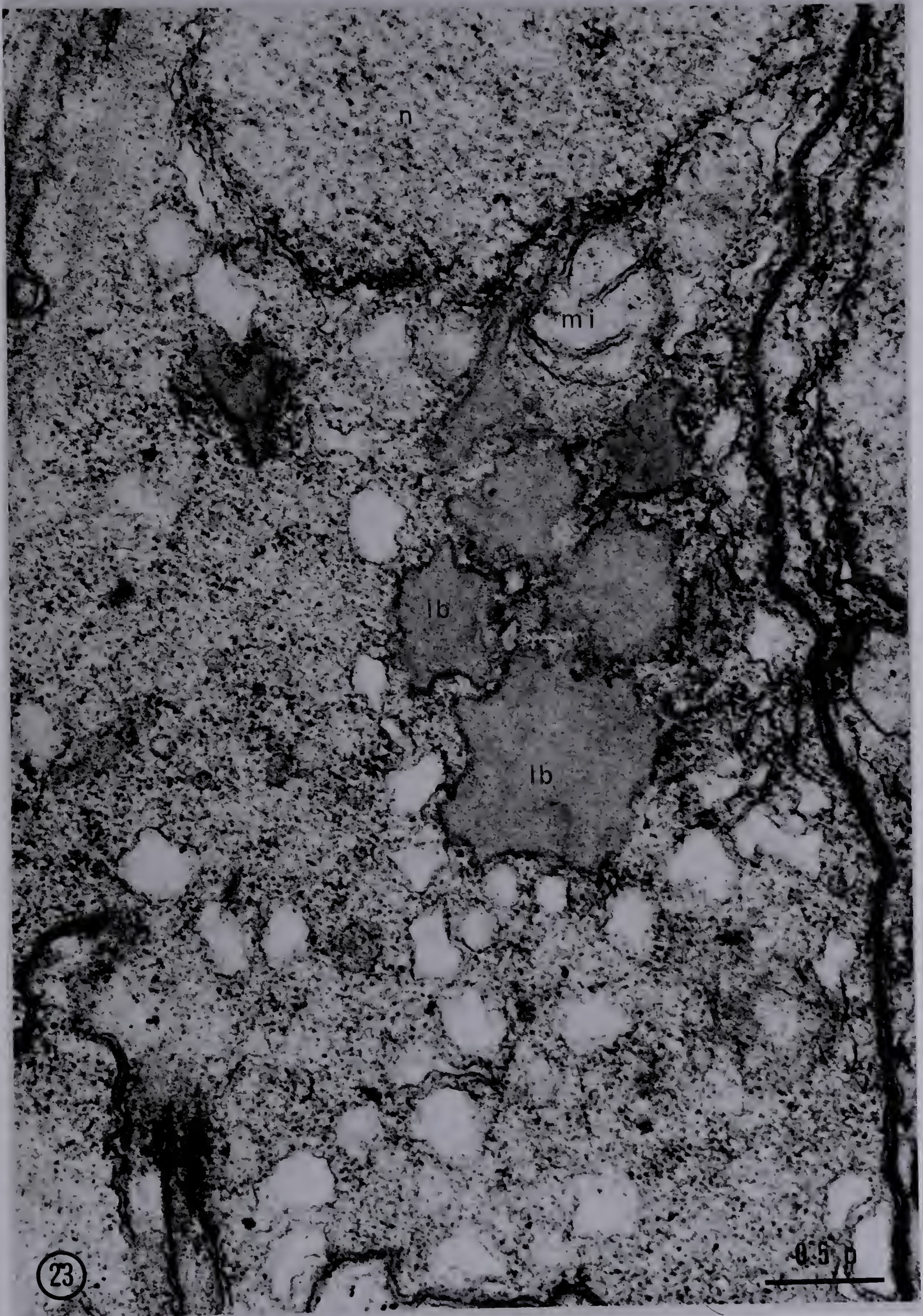


PLATE 24. Section through apical portion of proventricular epithelial cell. Note microvilli (mv) projecting from surface cell membrane (scm) into lumen of gut. pv, 'pinocytotic' vesicle.

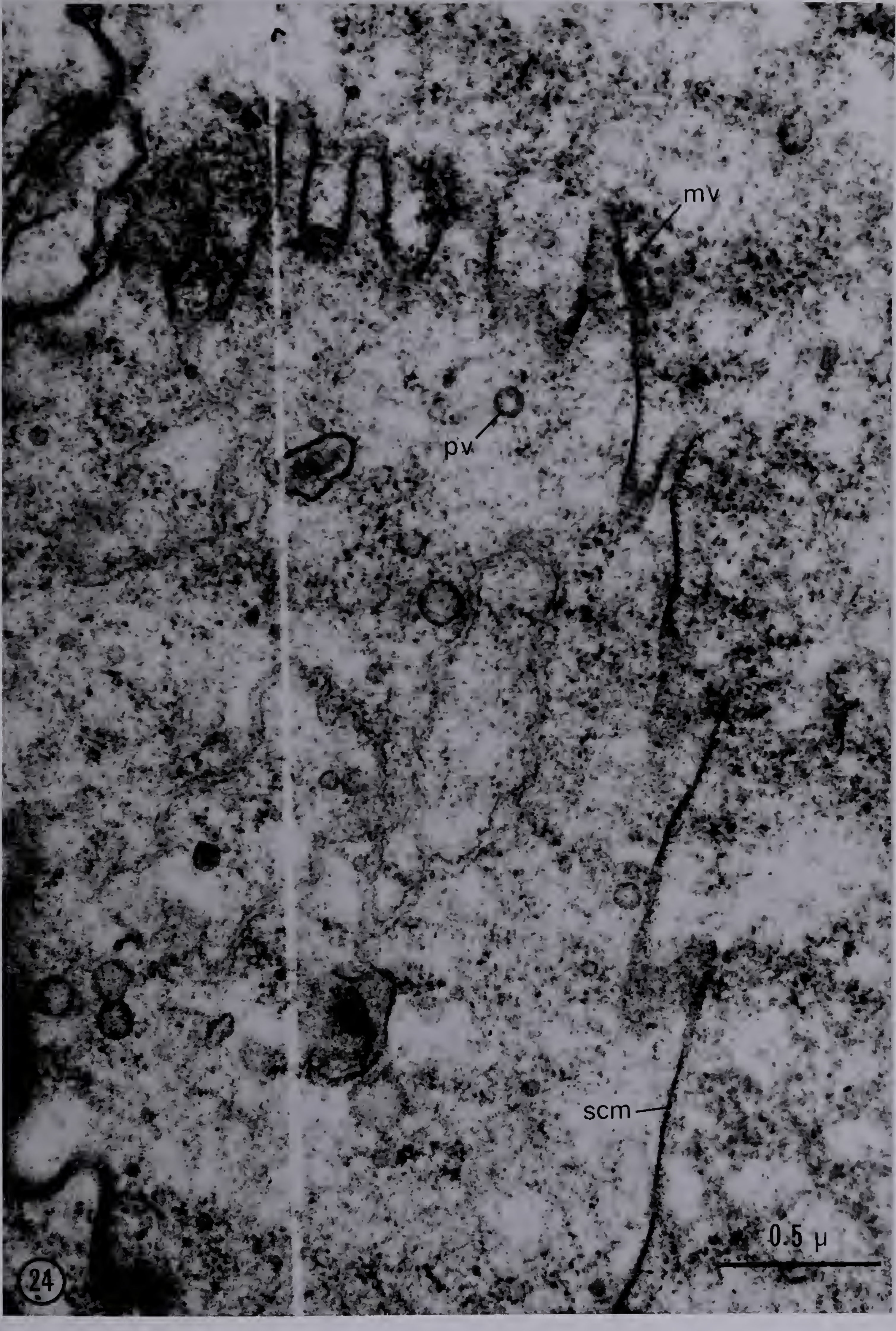

mv
pv
scm
0.5 µ
24

PLATE 25. Distal portion of proventricular epithelial cell, showing 'pinocytotic vesicles' (pv).
mvb, multi-vesicular body. scm, surface cell membrane.

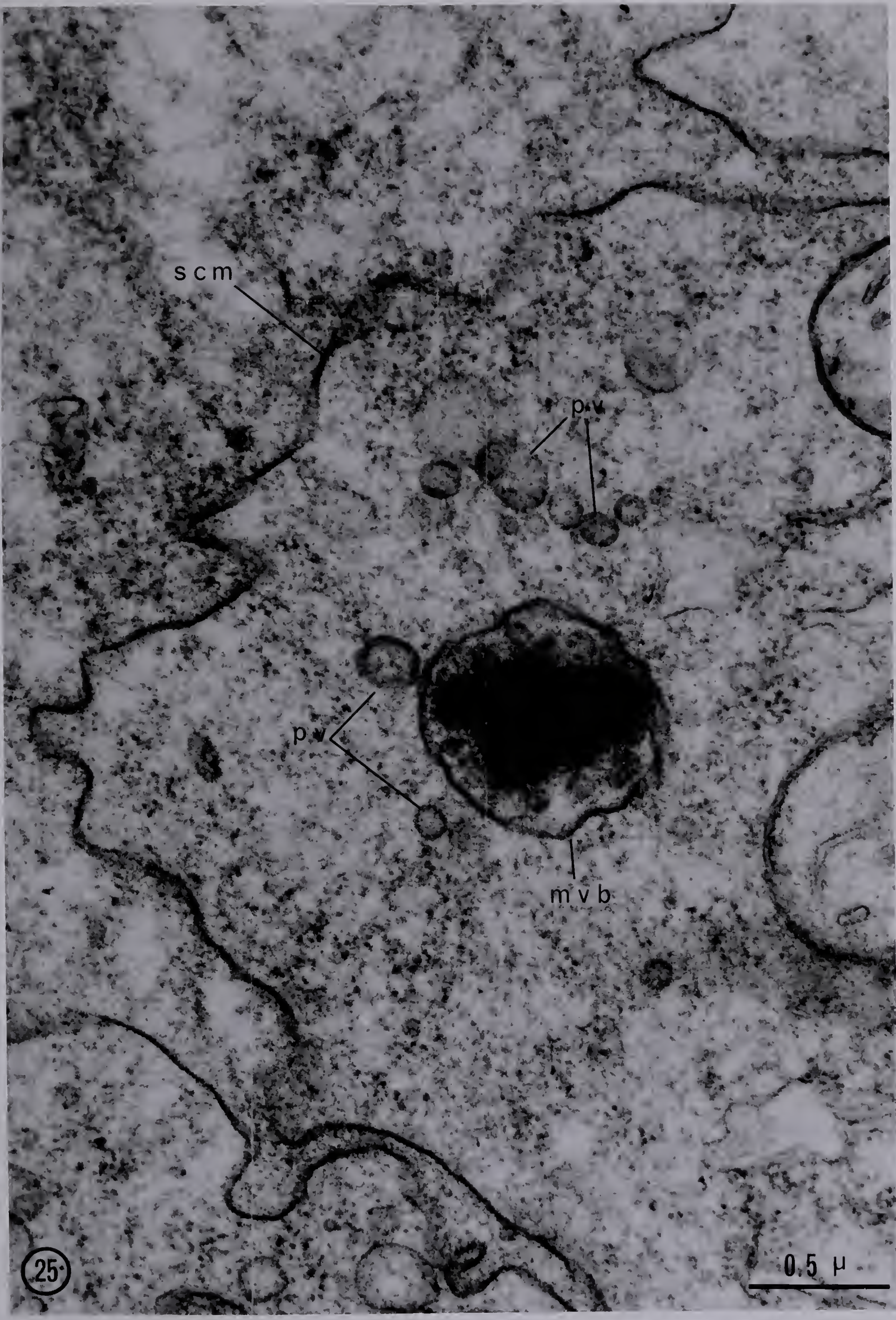

scm
pv
pv
mvb
25
0.5 μ

PLATE 26. Section through basal portion of proventricular cell. Note dense population of small vesicles with intense electron-dense core (arrow). cm, cell membrane. n, nucleus.

(26)

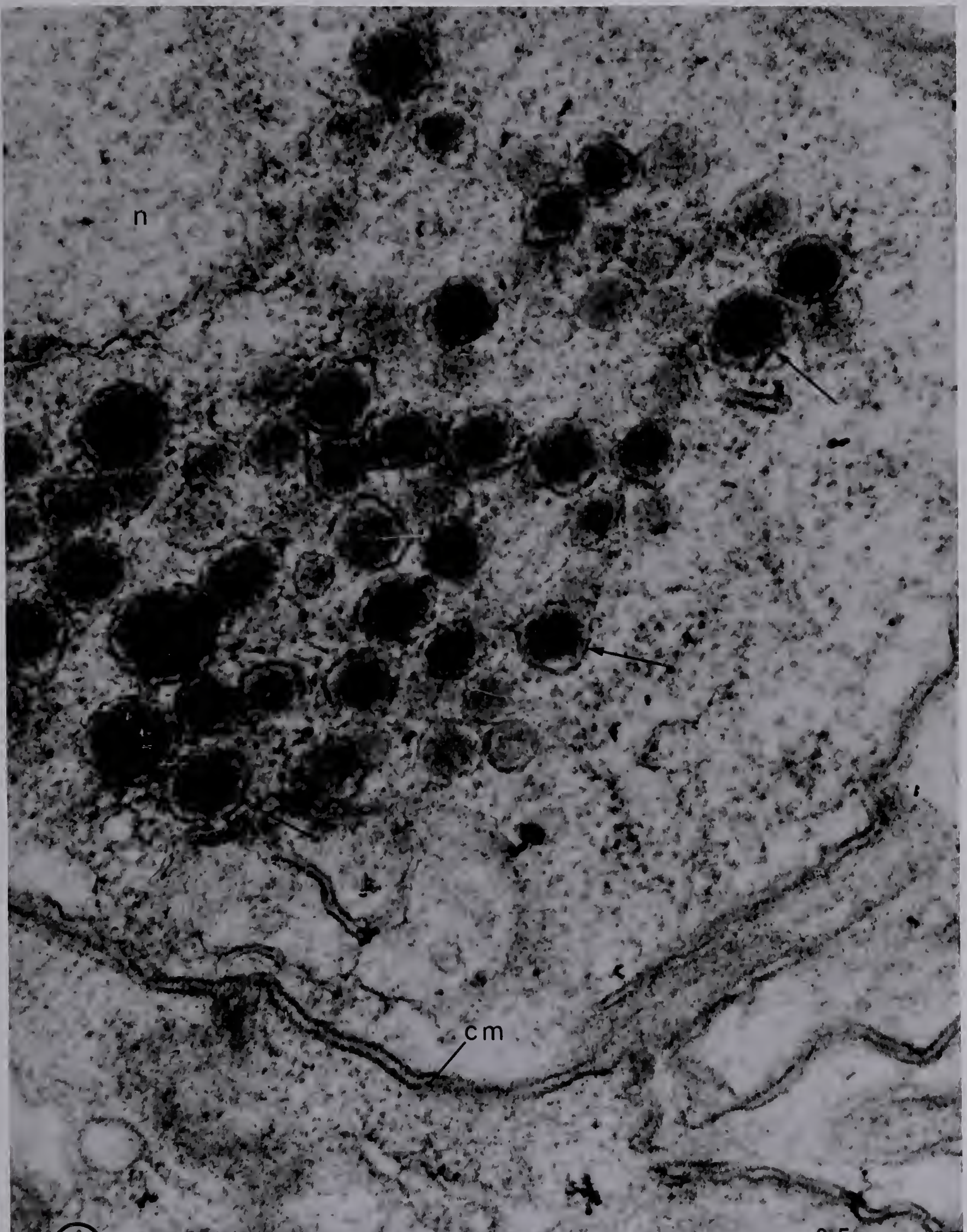


PLATE 27. Cross-section through several epithelial cells, showing densely packed rough endoplasmic reticulum (rer), and electron-dense granules (gr). Note dilated tips (arrows) of Golgi complex (Gc). lf, lamellate figure. mi, mitochondrion.

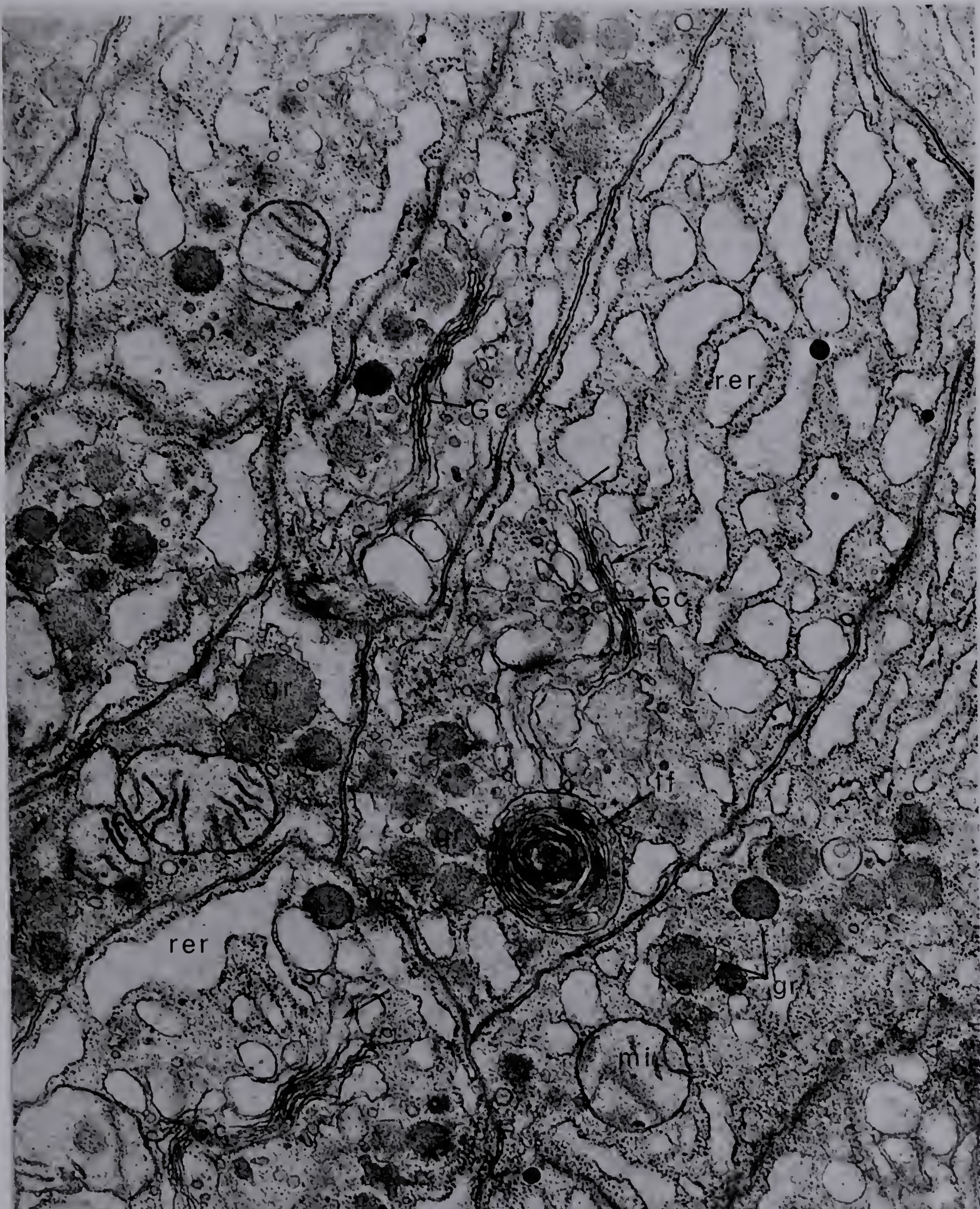


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PLATE 28. Section through nuclear region of stomach epithelial cell. Note dilated nuclear envelope (ne), with ribosomes arrayed along the outer membrane (r). Free ribosomes and polysomes (cross-hatched arrow) lie scattered throughout the cell.

gr, electron-dense granule. np, nuclear pore.

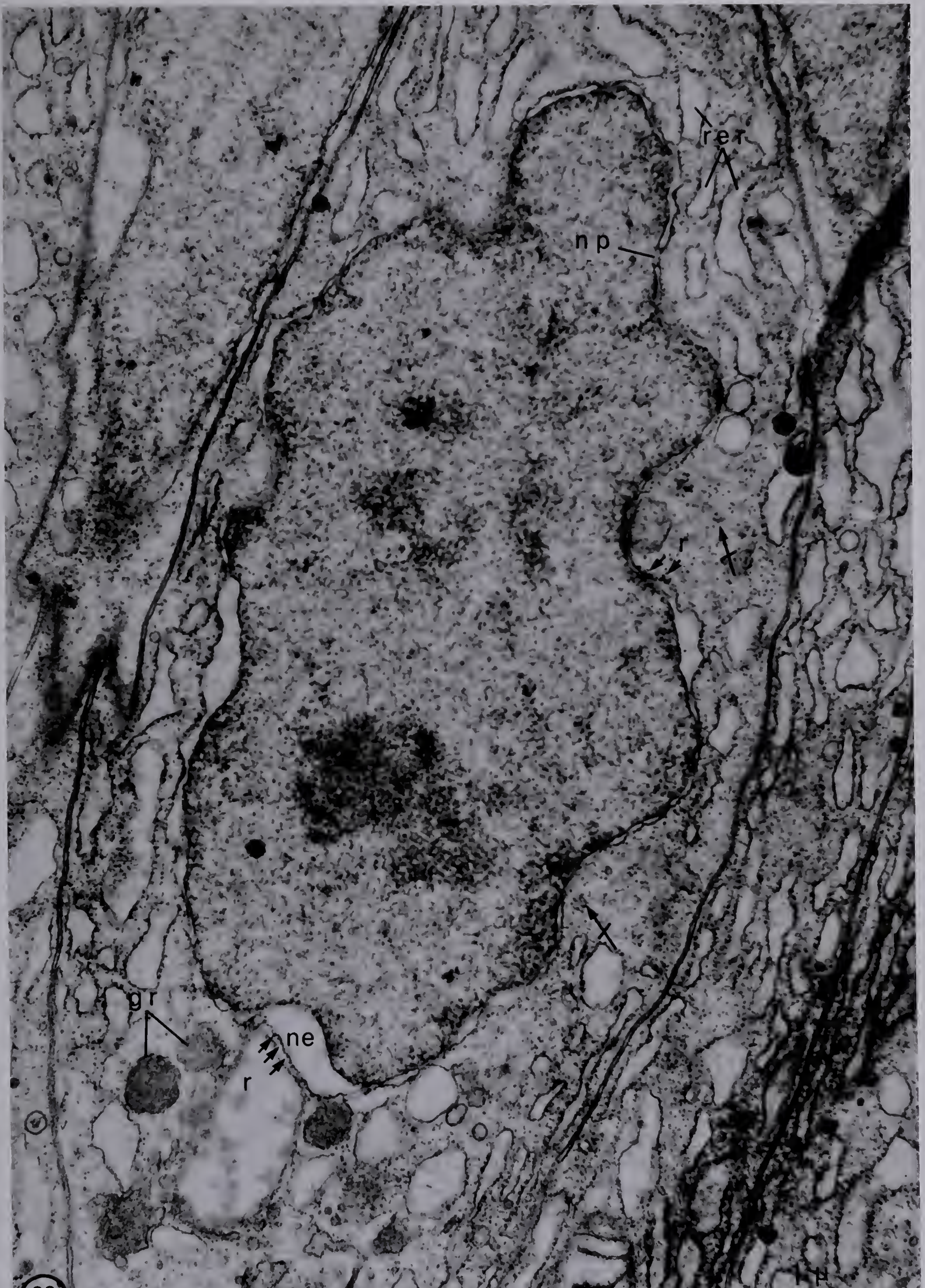

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PLATE 29. Mitochondria (mi) in intimate association with elements of rough endoplasmic reticulum (rer) and electron-dense granules (gr). Note free ribosomes (arrows).

29

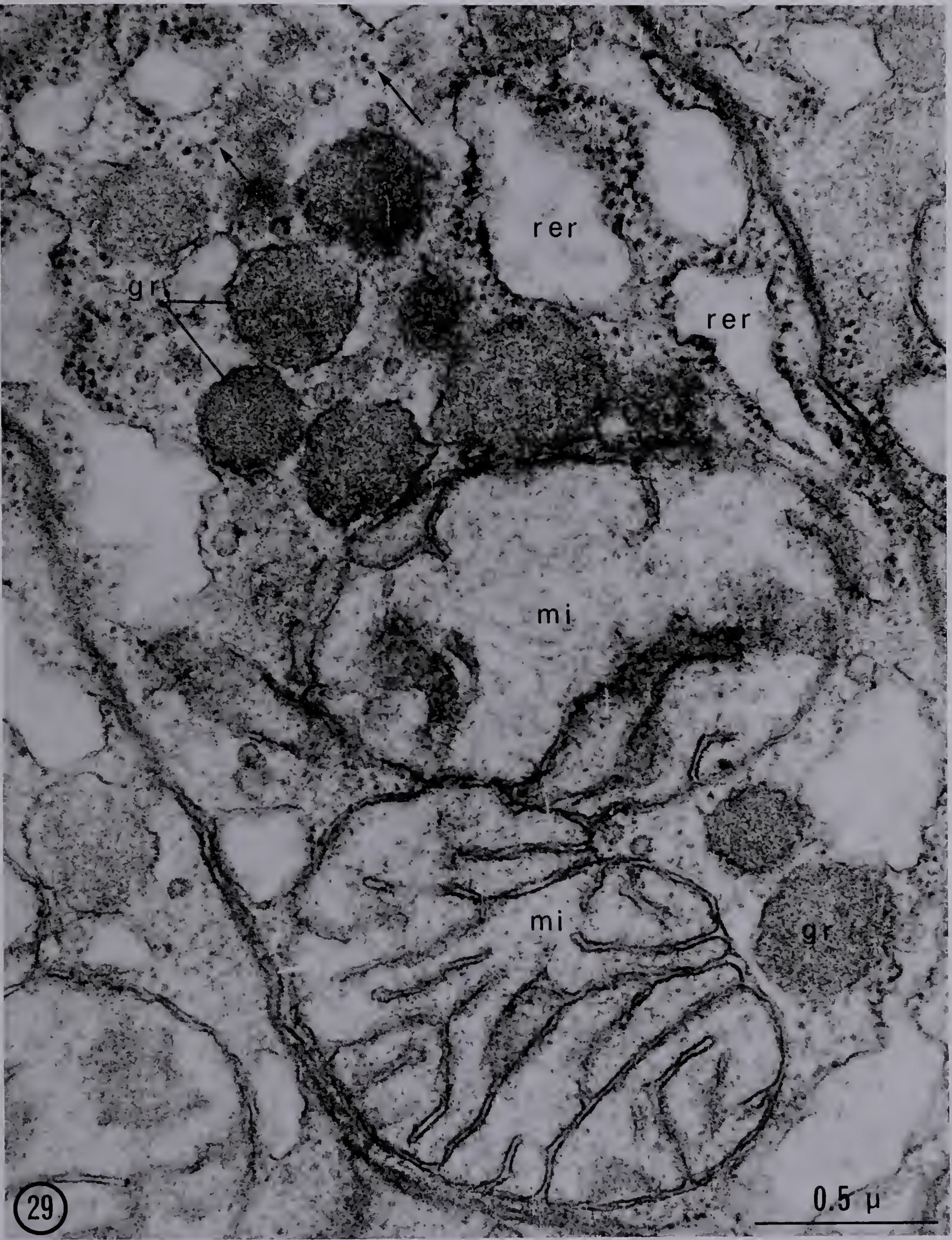

PLATE 30. Cross-section through stomach epithelial cell. Note Golgi complex (Gc) in close association with numerous small vesicles (v) and electron-dense granules (gr).

n, nucleus. rer, rough endoplasmic reticulum.

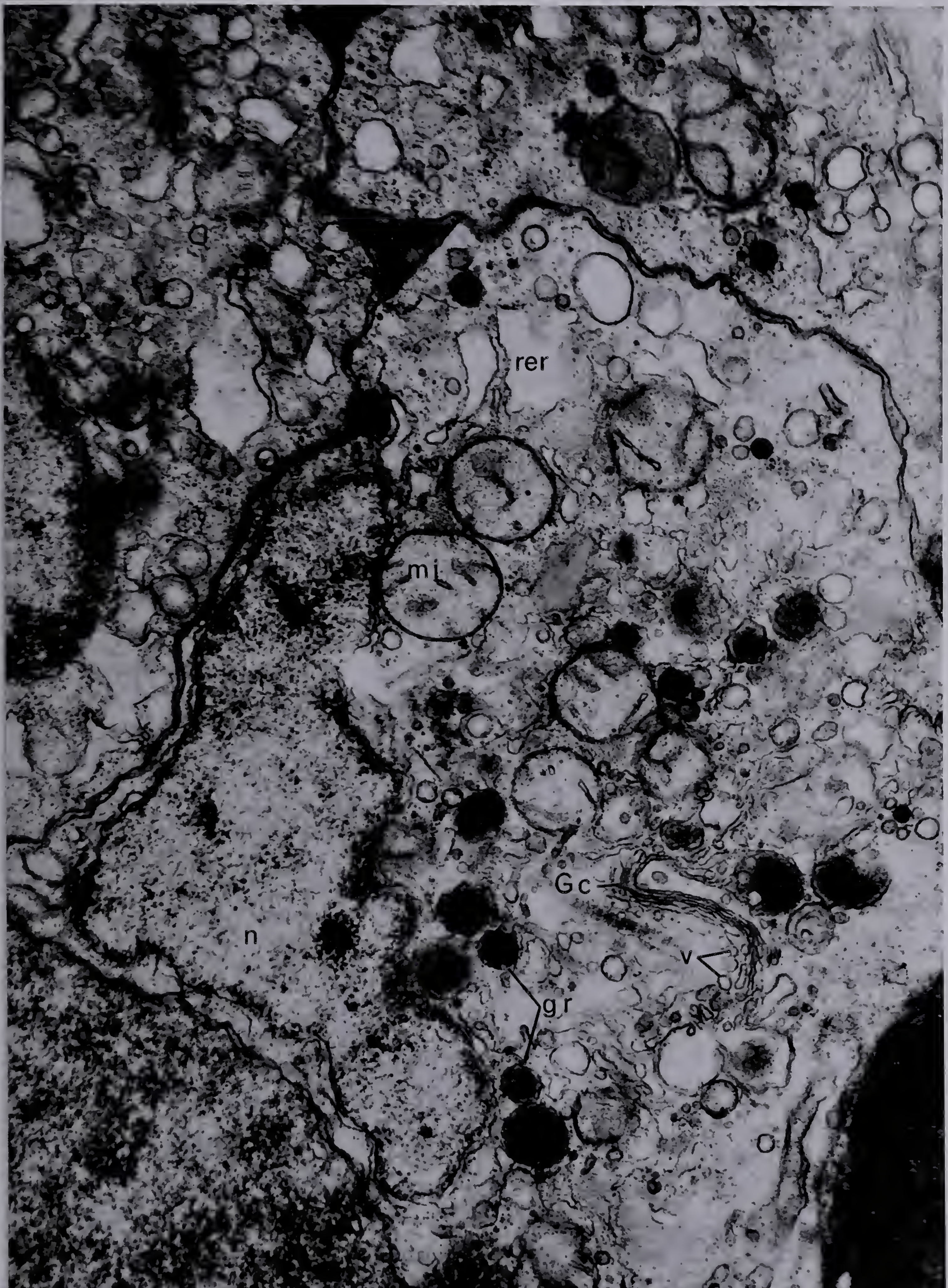

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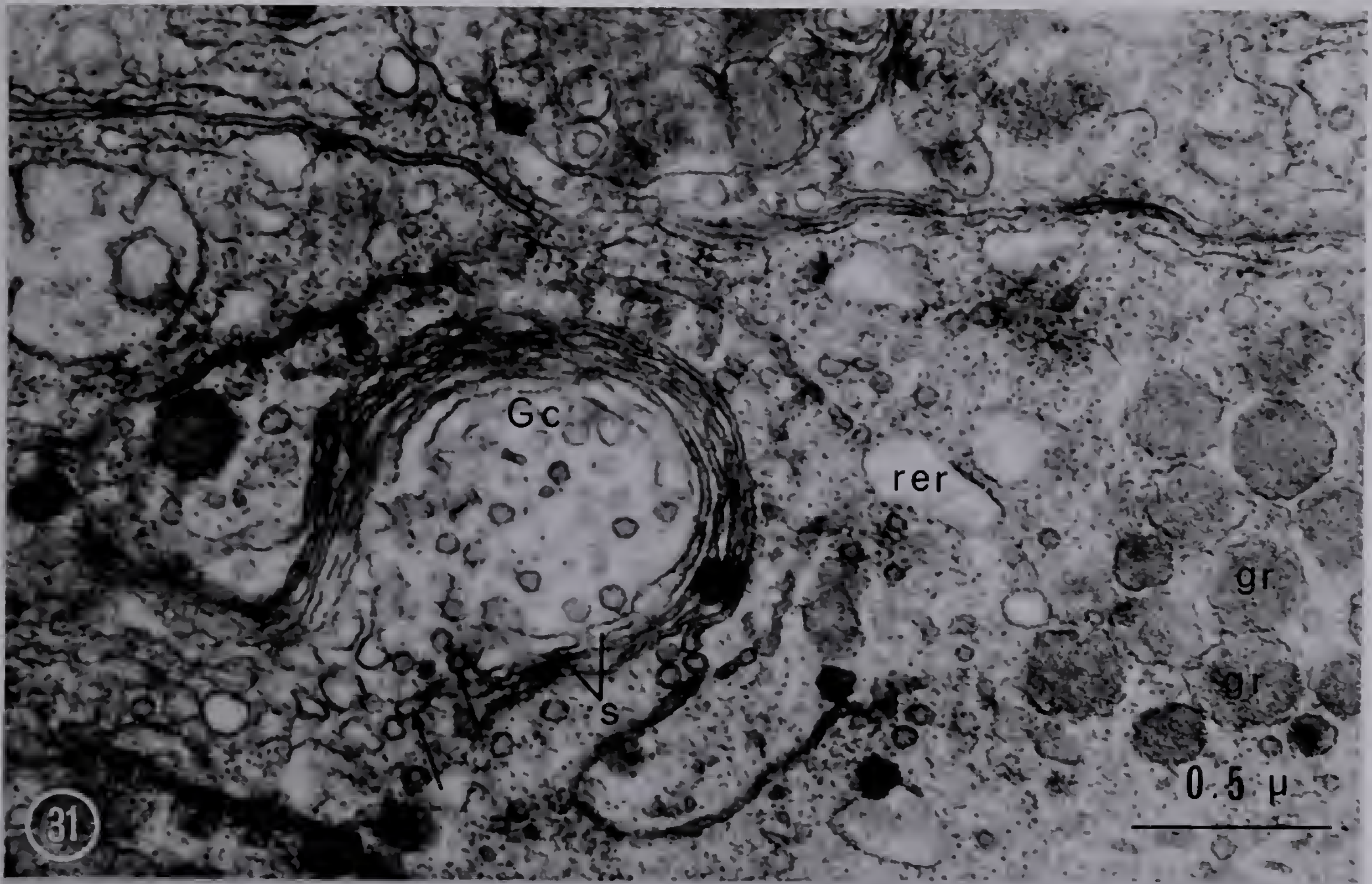
PLATE 31. Golgi complex in a stomach epithelial cell. Polarity of the complex is clearly evident in the thin outer cisternae and the distended inner cisternae. Tips of inner cisternae are swollen into saccules (s), and appear to be pinched off as individual vesicles (arrows) into the cytoplasm.

gr, electron-dense granules. rer, rough endoplasmic reticulum.

PLATE 32. Electron micrograph of luminal content from anterior portion of intestine. Note whorls (w) and membrane figures and amorphous electron dense masses (arrows).


Gc
rer
gr
gr
s
0.5 μ
31

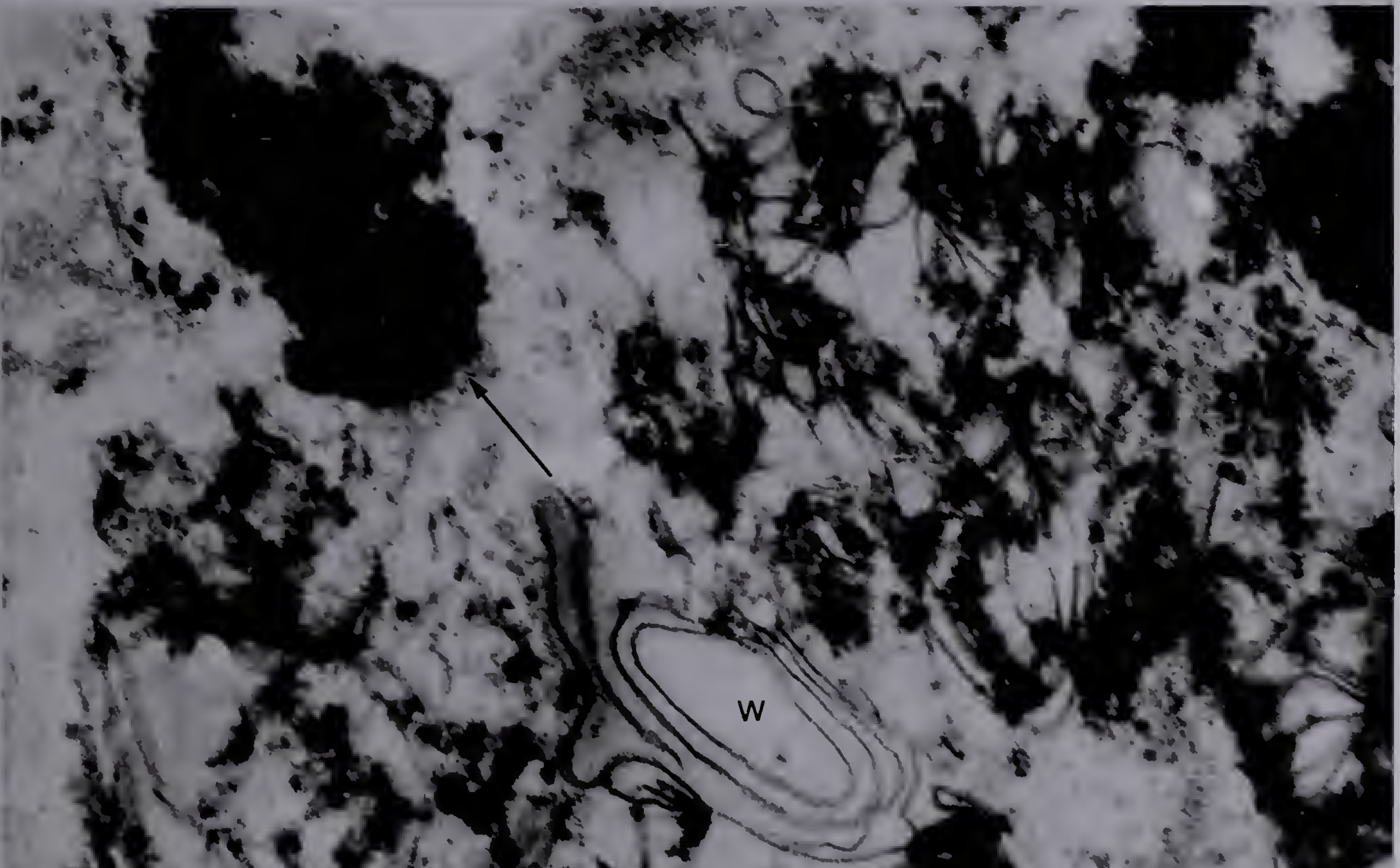

w
1 μ
32

PLATE 33. Section through anterior portion of intestinal epithelium, showing ciliary rootlet with characteristic cross-striation of two wide bands and two narrow bands. Note mitochondrion (mi) closely associated (arrow) with rootlet (rtlt).
gr, electron-dense granule. mvb, multi-vesicular body.

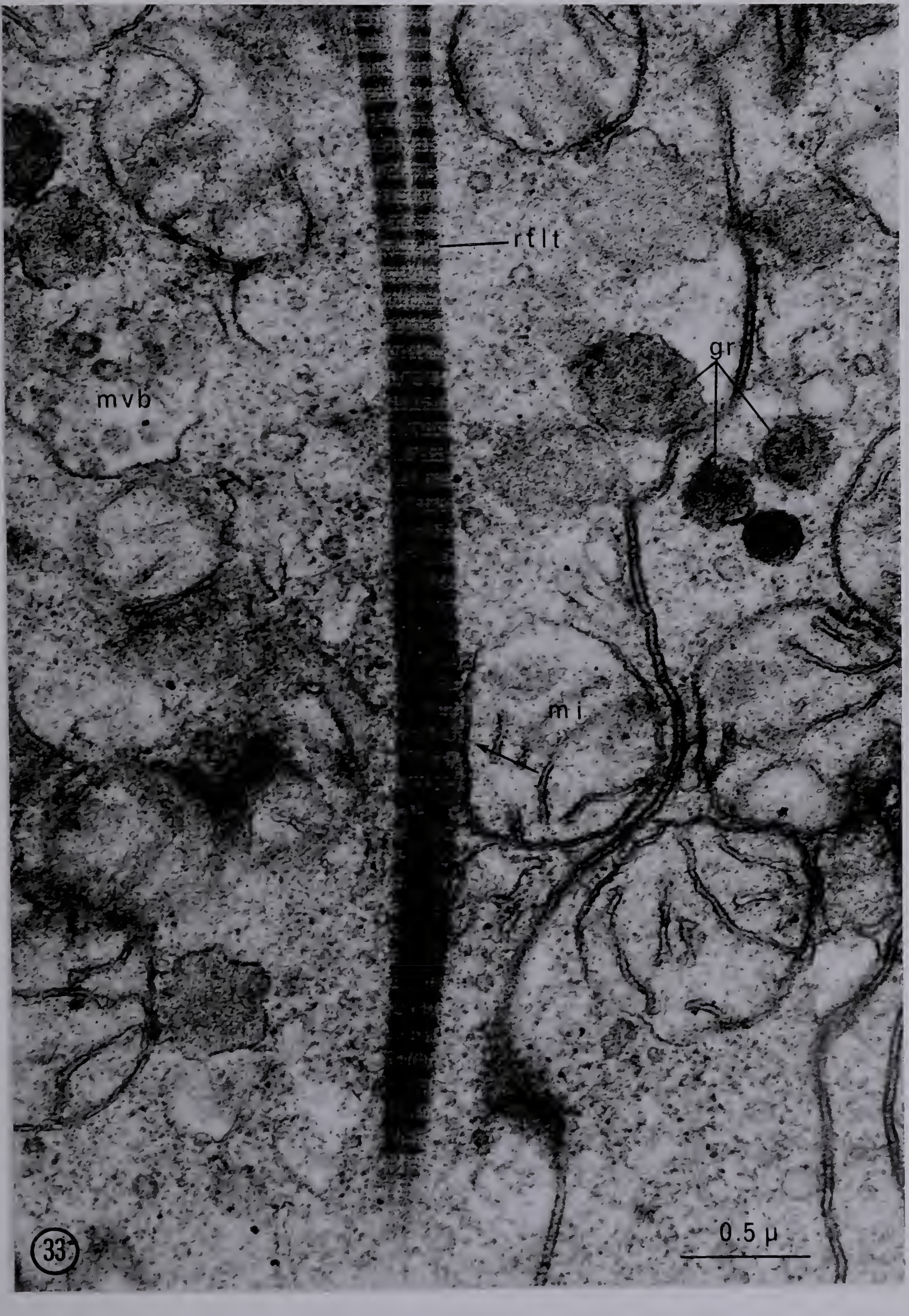

rflt
gr
mvb
mi
0.5 μ
33

PLATE 34. Transverse section through epithelium in anterior portion of intestine. Note paired cilia enveloped basally within a common membrane (cm). Note also the paired basal bodies within the cell cytoplasm (bb).

er, endoplasmic reticulum.

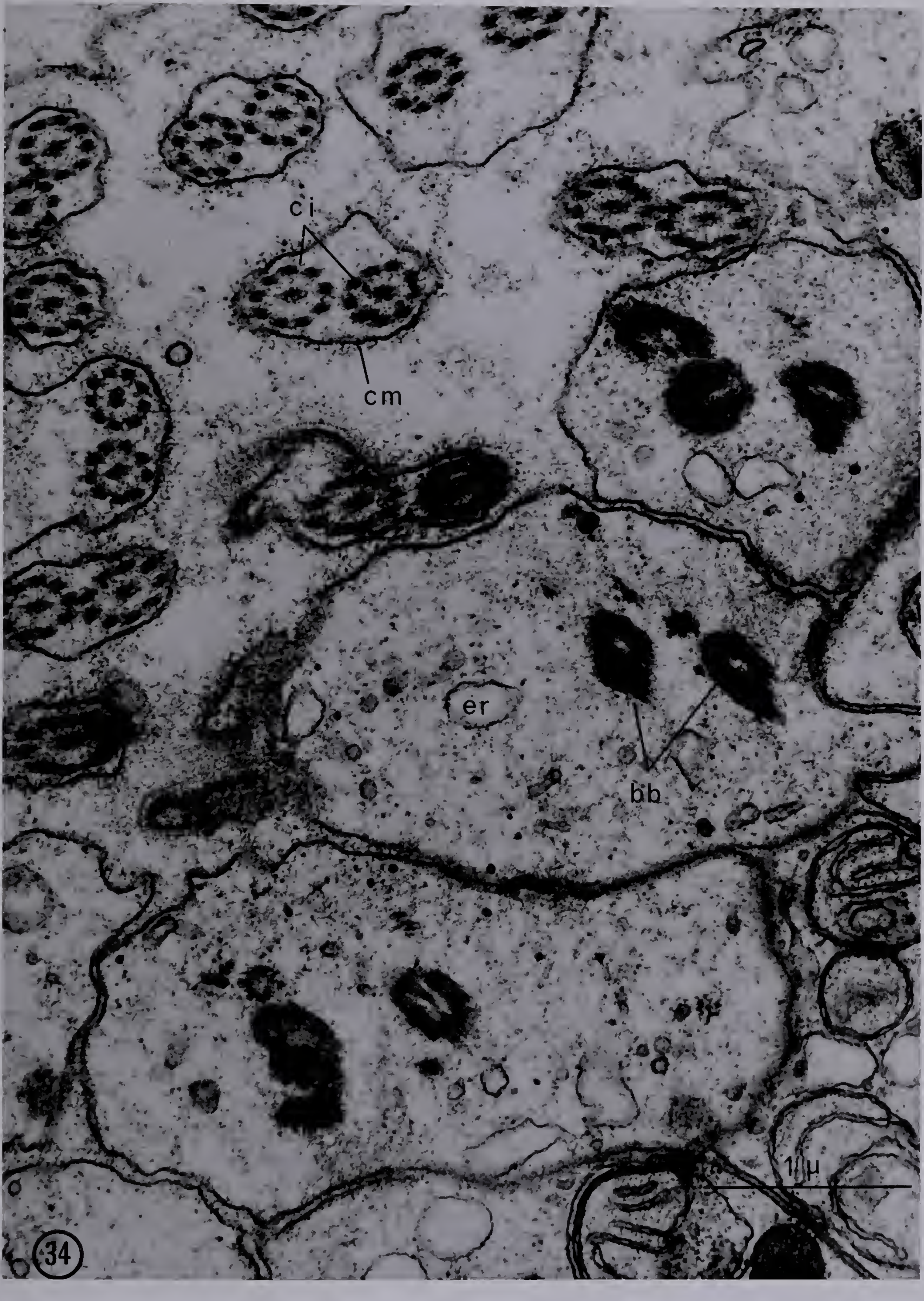

ci
cm
er
bb
1 μ
34

PLATE 35. Longitudinal section through ciliated intestinal epithelium cells. The fibrils terminate in a basal plate (bp), separated from the basal body (bb) by a brief clear space (arrow). Note the characteristic cross-striation periodicity of the rootlet (rtlt) and the second basal body (bb2) lying at right angles to the first basal body. Mitochondria lie in intimate association with ciliary rootlets (mi).

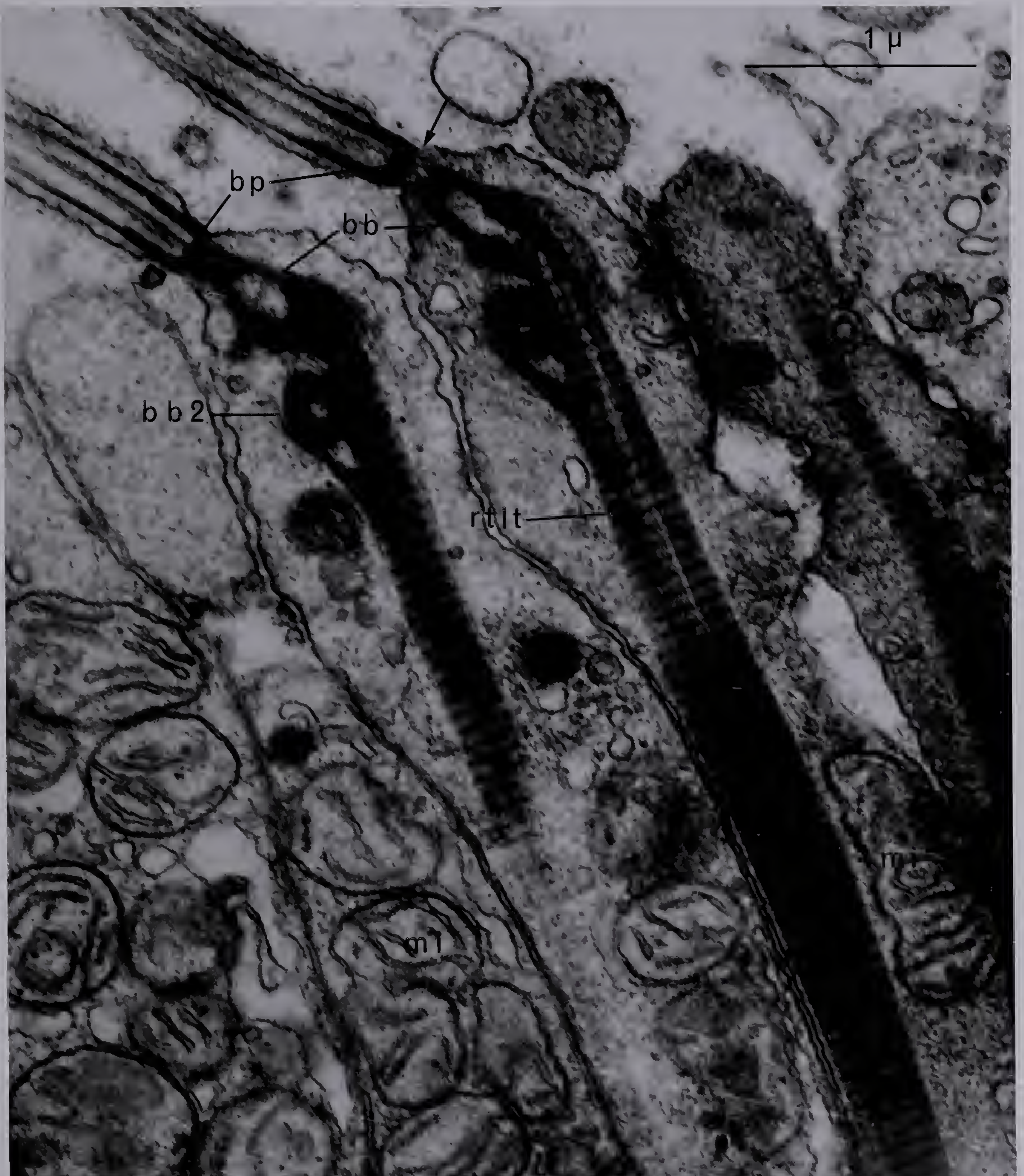


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PLATE 36. Mucus secreting cell in intestinal epithelium. Note the compacted mass of secretory globules (g).

bm, basement membrane. lb, lipid bodies.

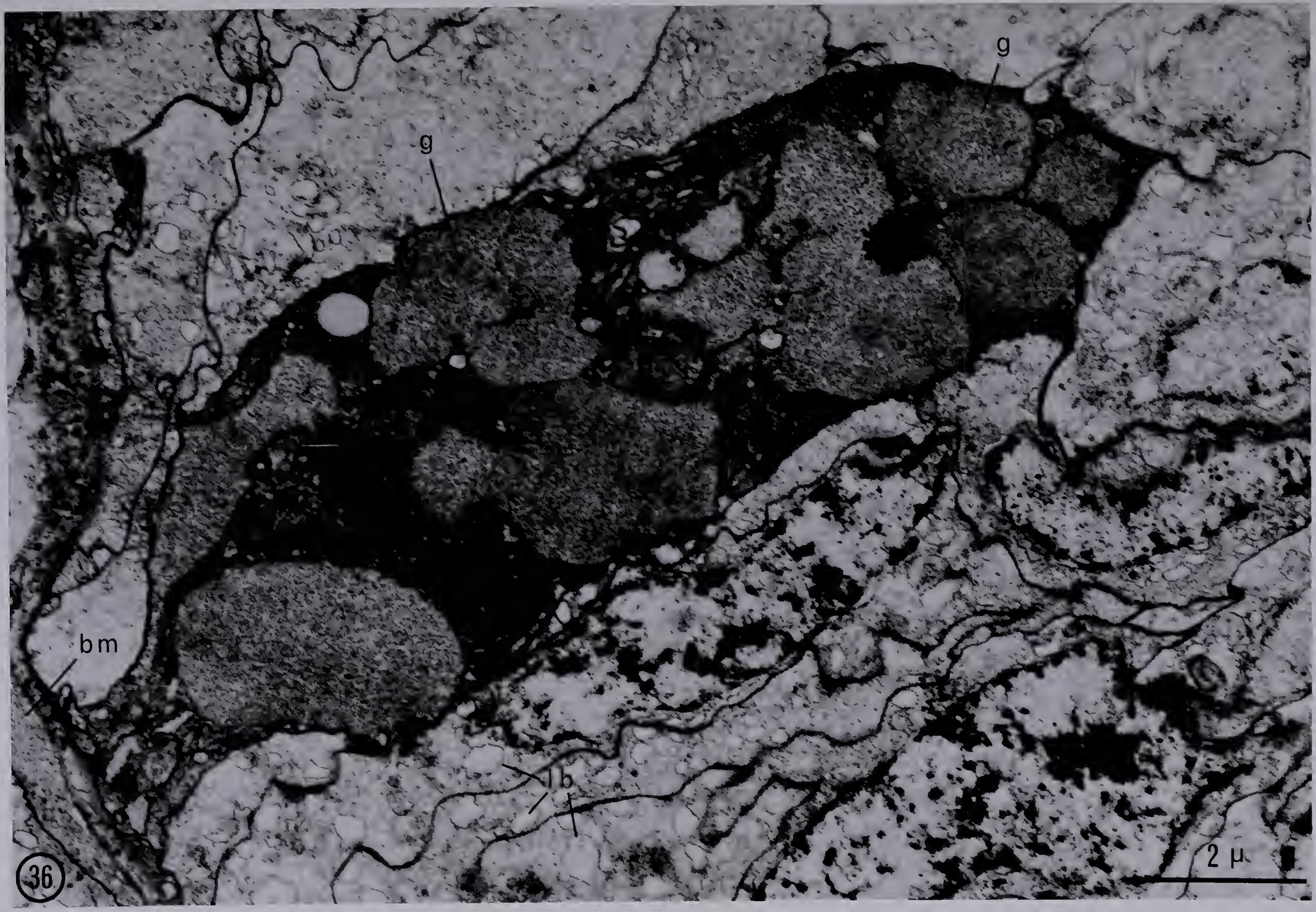

g
g
bm
lb
36
2 μ

PLATE 37. 'Dark cell' in intestinal epithelium. Note wrinkled appearance of mitochondria (mi) and the larger numbers of this organelle as compared with the adjacent lighter cells.

ci, cilium. er, endoplasmic reticulum.

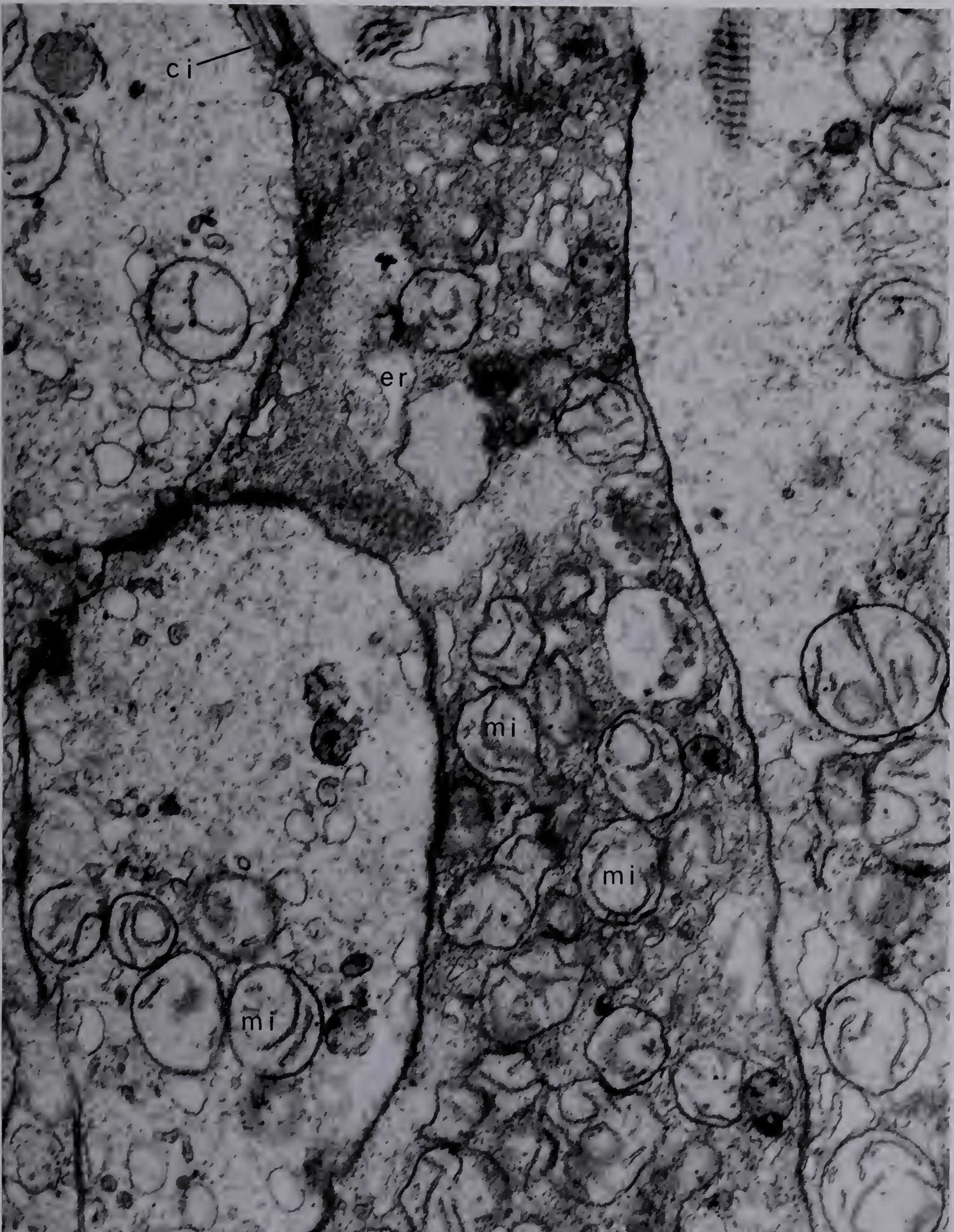

ci
er
mi
mi
mi
37
1 µ

PLATE 38. Transverse section through ciliated epithelial fold in intestine. The double nature of the peripheral fibrils in the cilium (ci) is clearly evident. Note absorptive vesicles (av) and dark core structures (dcs) in apical cytoplasm of epithelial cells.

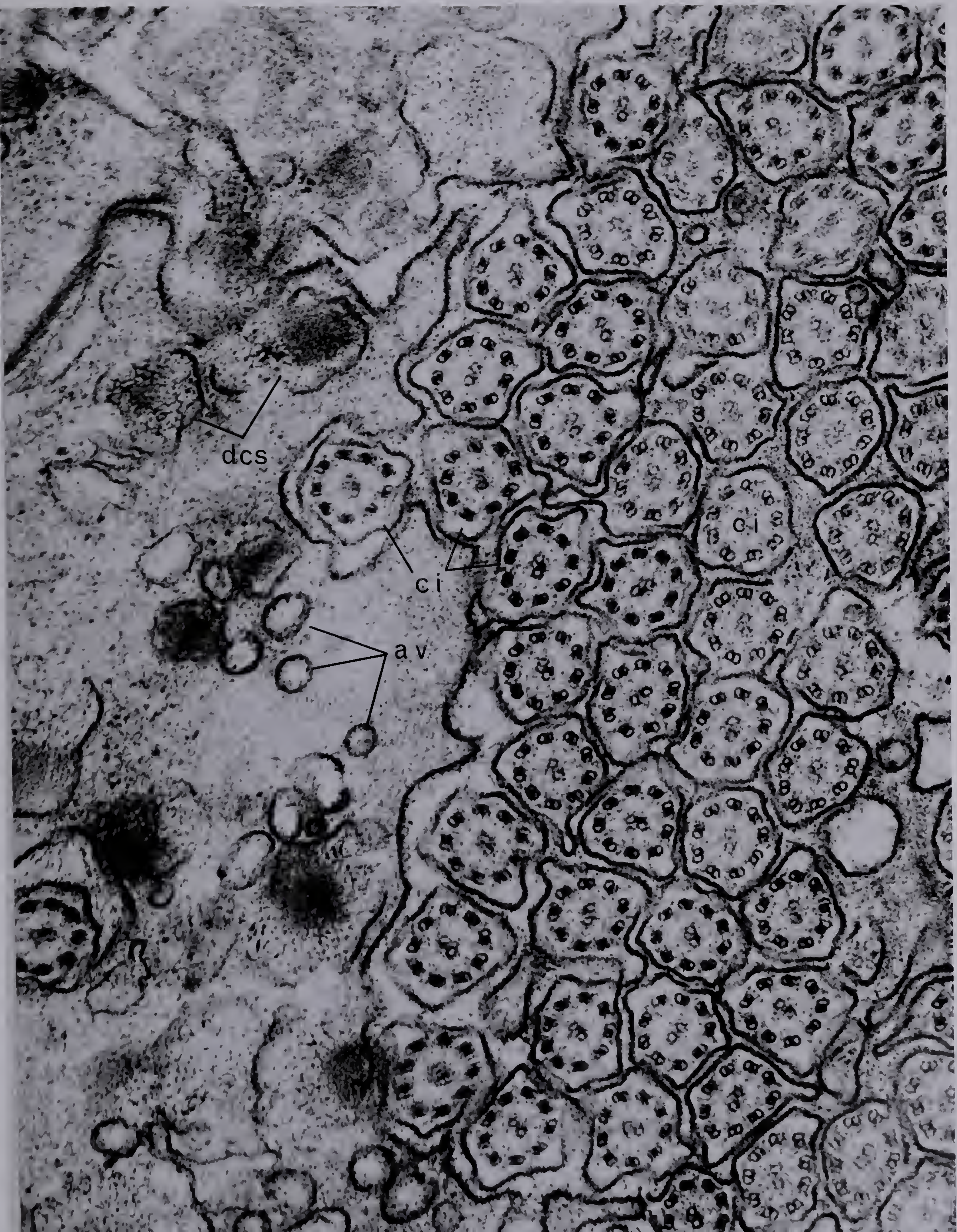

dcs
ci
ci
av
38
1 μ

PLATE 39. Section through intestinal epithelium showing microvilli (mv) projecting into lumen of gut. Note coat material radiating out from the microvilli (arrows).

dcs, dark core structure.

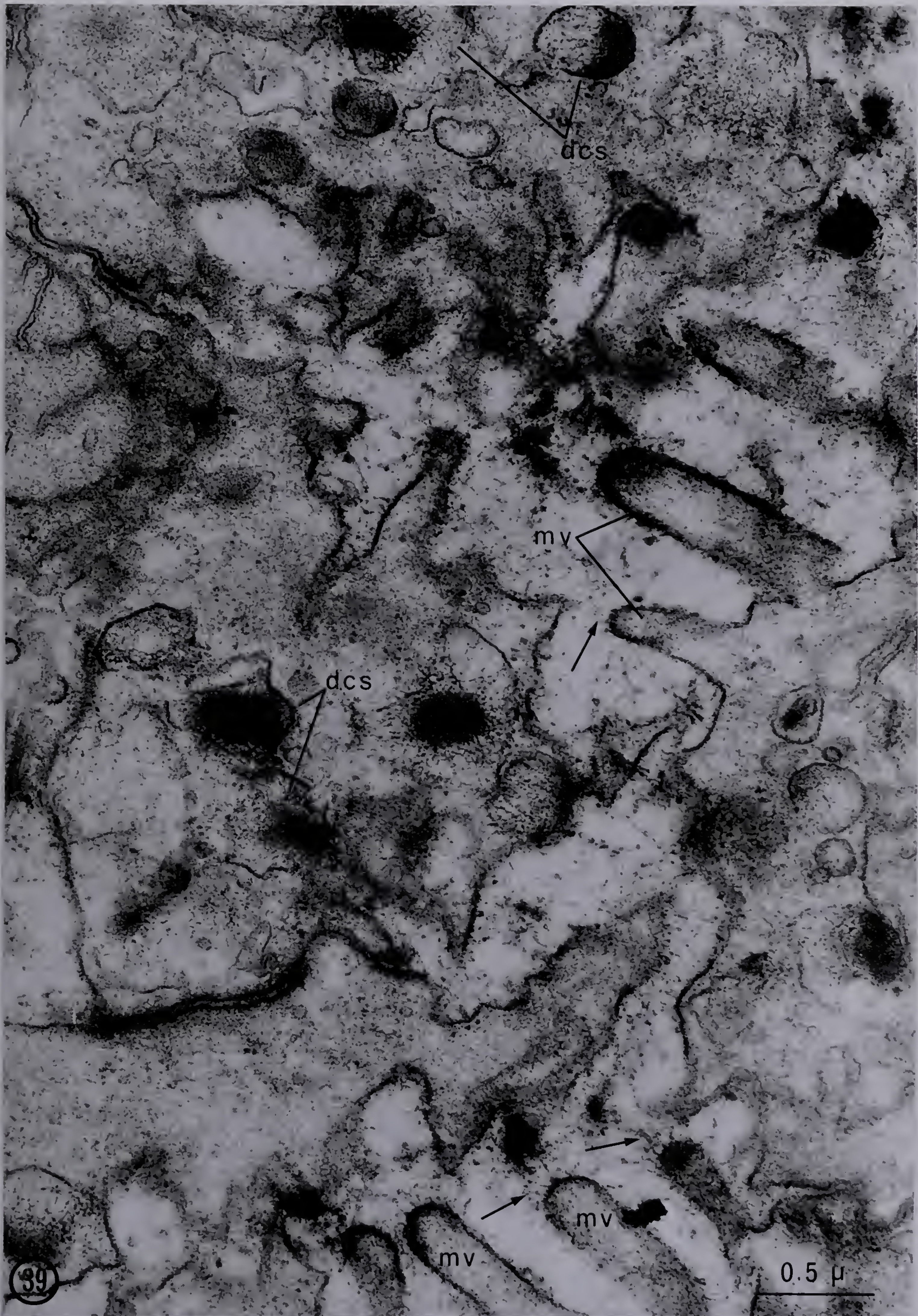

dcs
mv
dcs
mv
mv
0.5 μ
39

PLATE 40. Section through intestinal epithelium cells, showing ciliation (ci), and single-membrane bounded structures with electron-dense contents (dcs). Note absorptive vesicles (av), some with conspicuous layer of coat material adherent to inner side of limiting membrane.

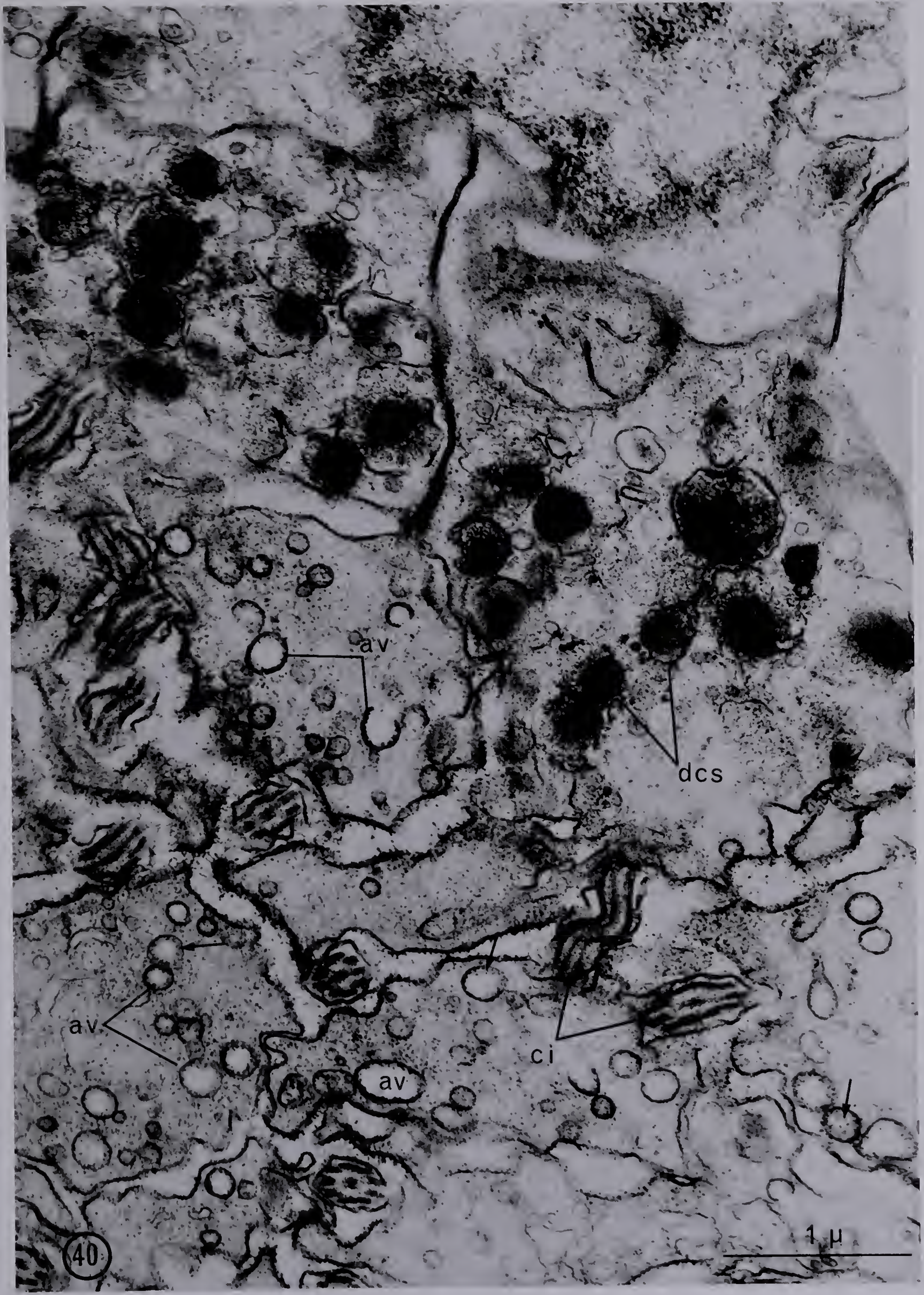
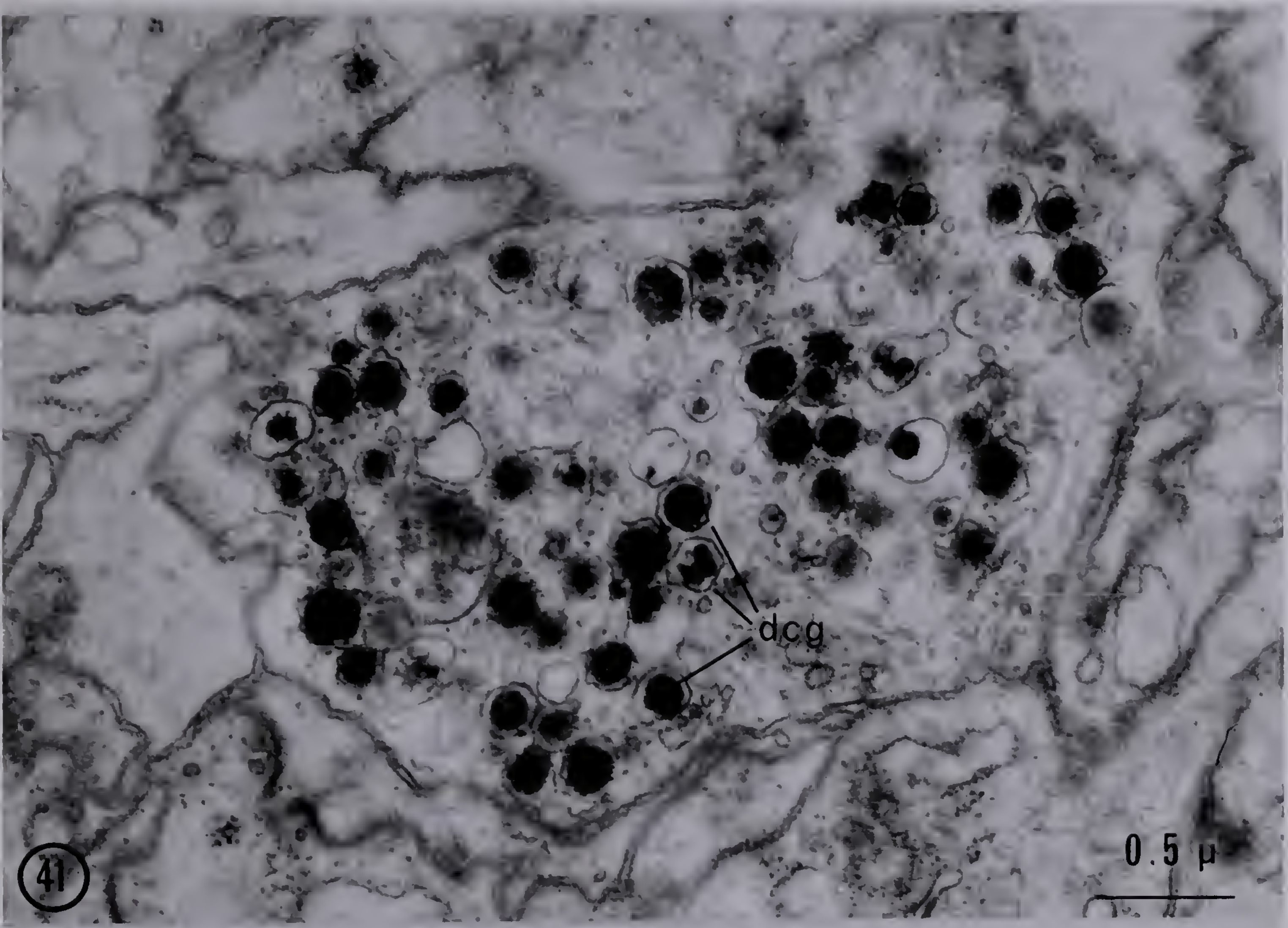

av
dcs
av
ci
av
40
1 μ

PLATE 41. Section through basal region of intestinal cell. Note the small dense core granules (dcg).

PLATE 42. Section through non-cellular mass lying in close proximity to the basement membrane (bm) in the intestinal epithelium.



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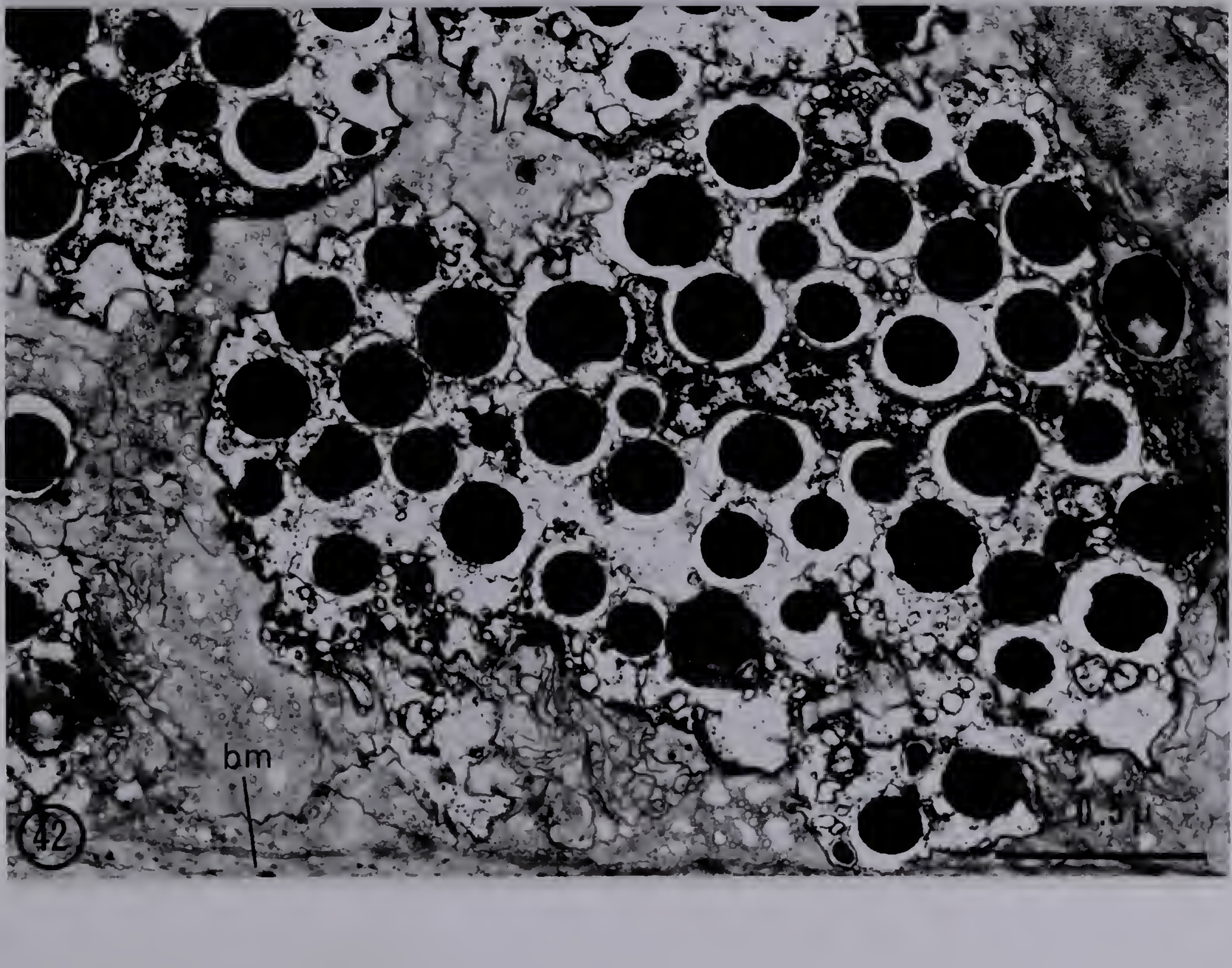

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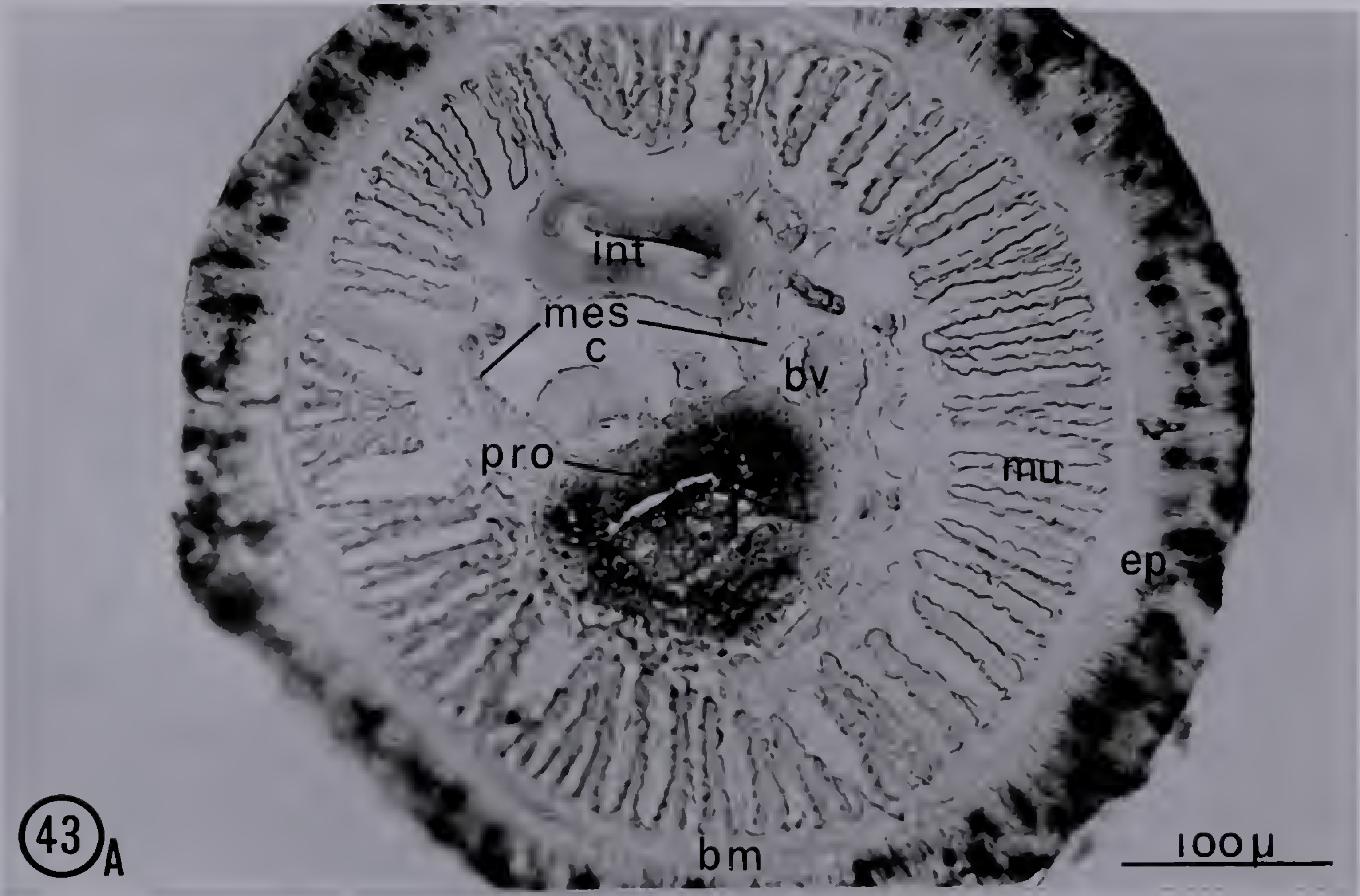
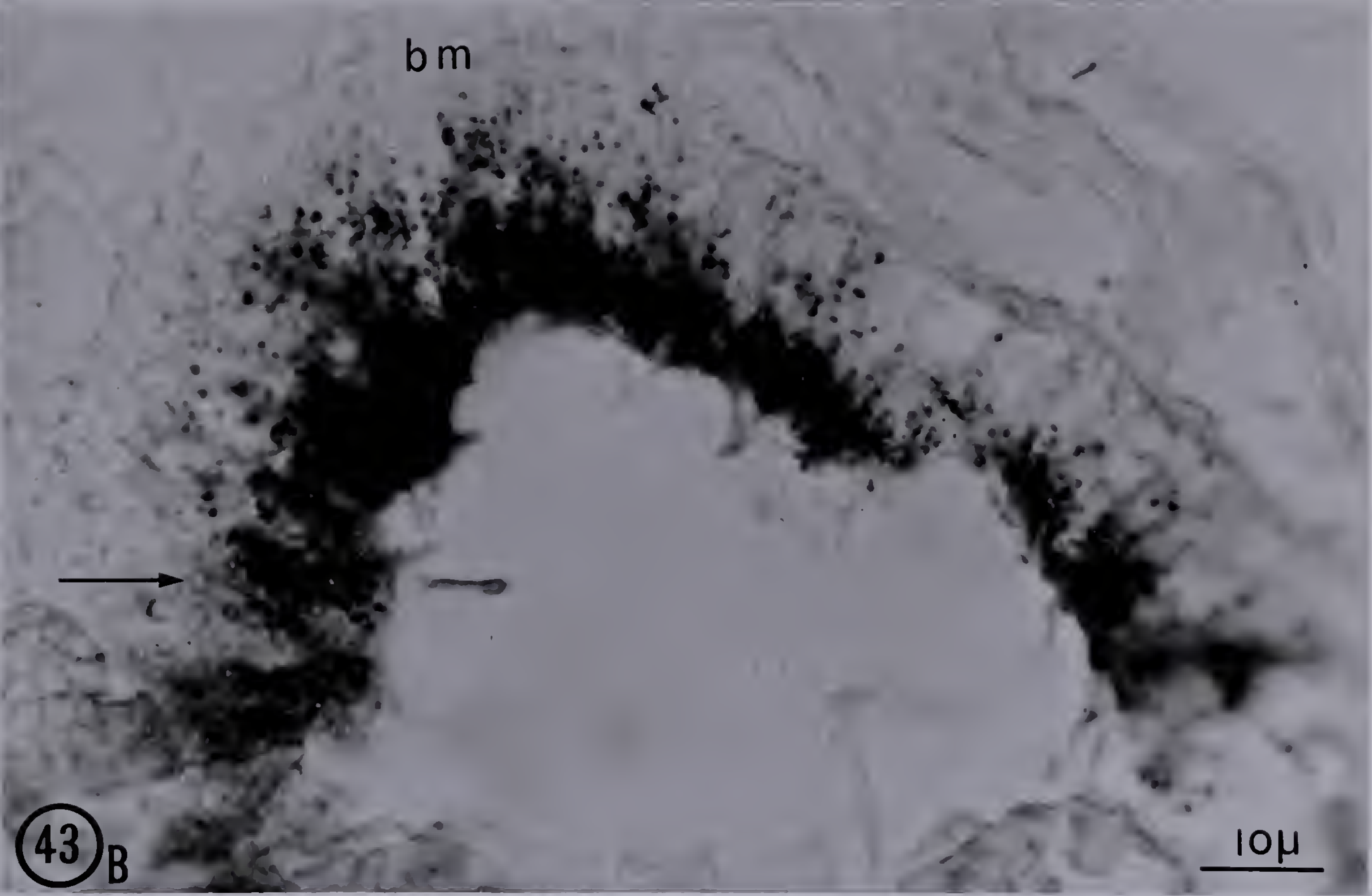
PLATE 43. Tissue stained for esterase activity. A, cross-section through proventriculus and intestine. B, section through proventricular epithelium. Esterase activity appears localized in globules, predominantly in apical cytoplasm, or in "esterase films" (arrows).

bm, basement membrane. bv, blood vessel. c, coelom. ep, epidermis. int, intestine. pro, proventriculus. Fresh frozen 15 μ sections. α-naphthyl acetate and Fast Blue B.



43 A

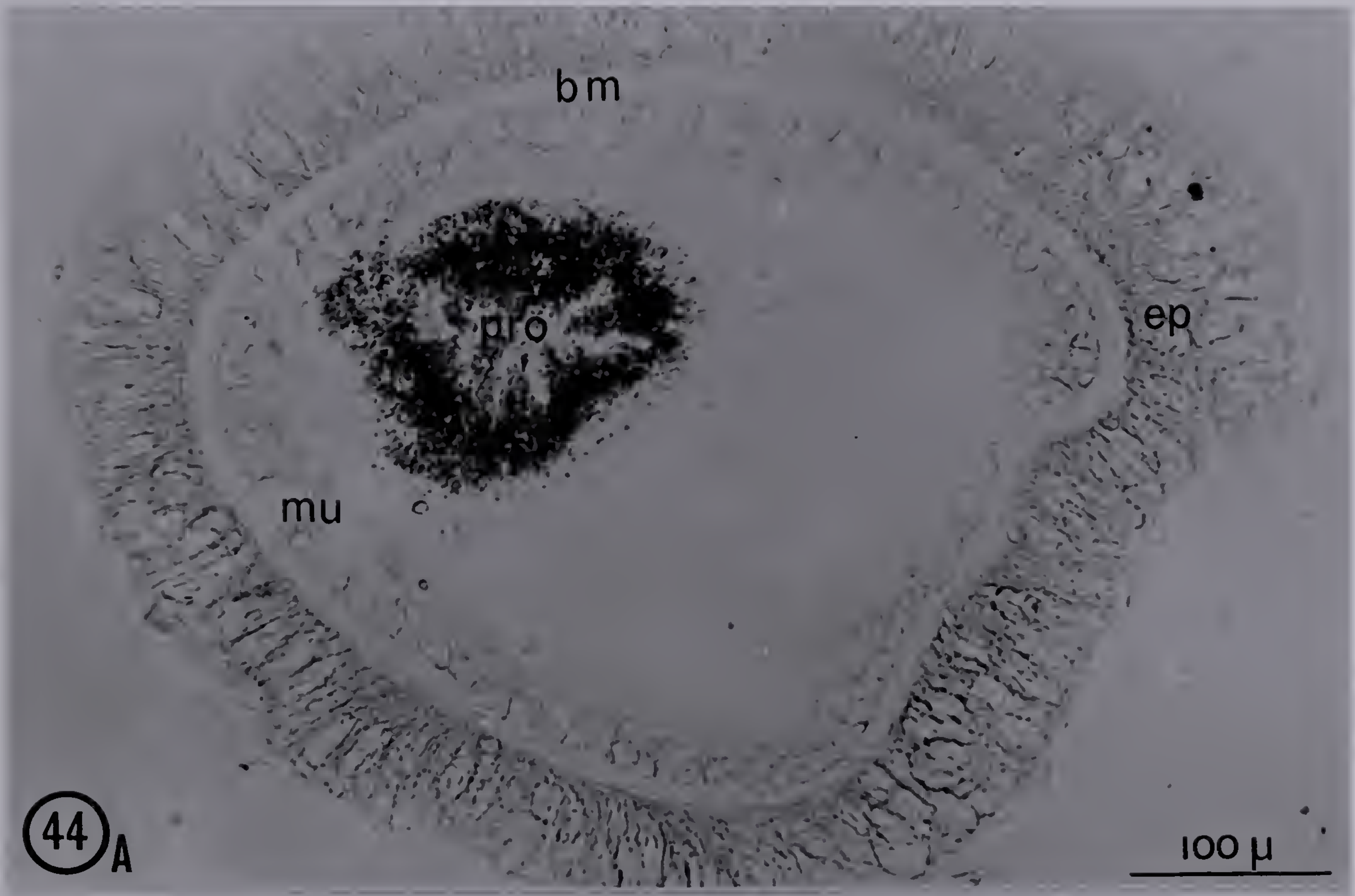


43 B

PLATE 44. Tissue stained for alkaline phosphatase activity. A, section through mid-trunk region, showing proventriculus (pro). Intestine not visible due to lack of stain uptake. B, high magnification of proventricular epithelium. Note film-like overlay of stain on globules in apical cytoplasm (arrows).

bm, basement membrane. ep, epidermis. mu, muscle.

Fresh frozen sections. α-naphthyl phosphate. pH 8.5 Garnet GBC.


bm
pro
ep
mu
44 A
100 µ

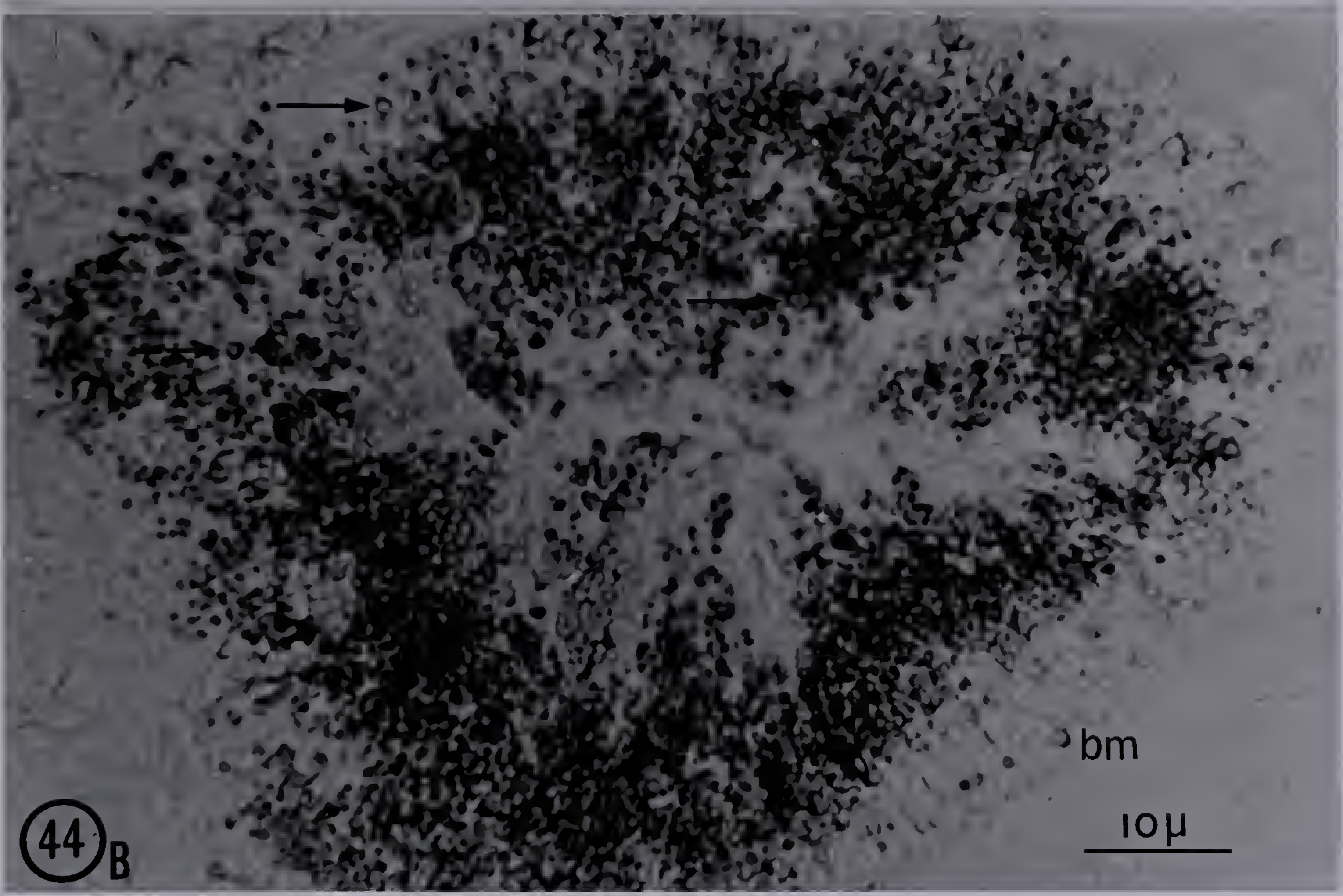

bm
44 B
10 µ

PLATE 45. Transverse section of mid-trunk region, showing acid phosphatase activity in proventricular and intestinal epithelial cells. Small, stain positive, globules (arrow) occur primarily in the proventricular epithelium.

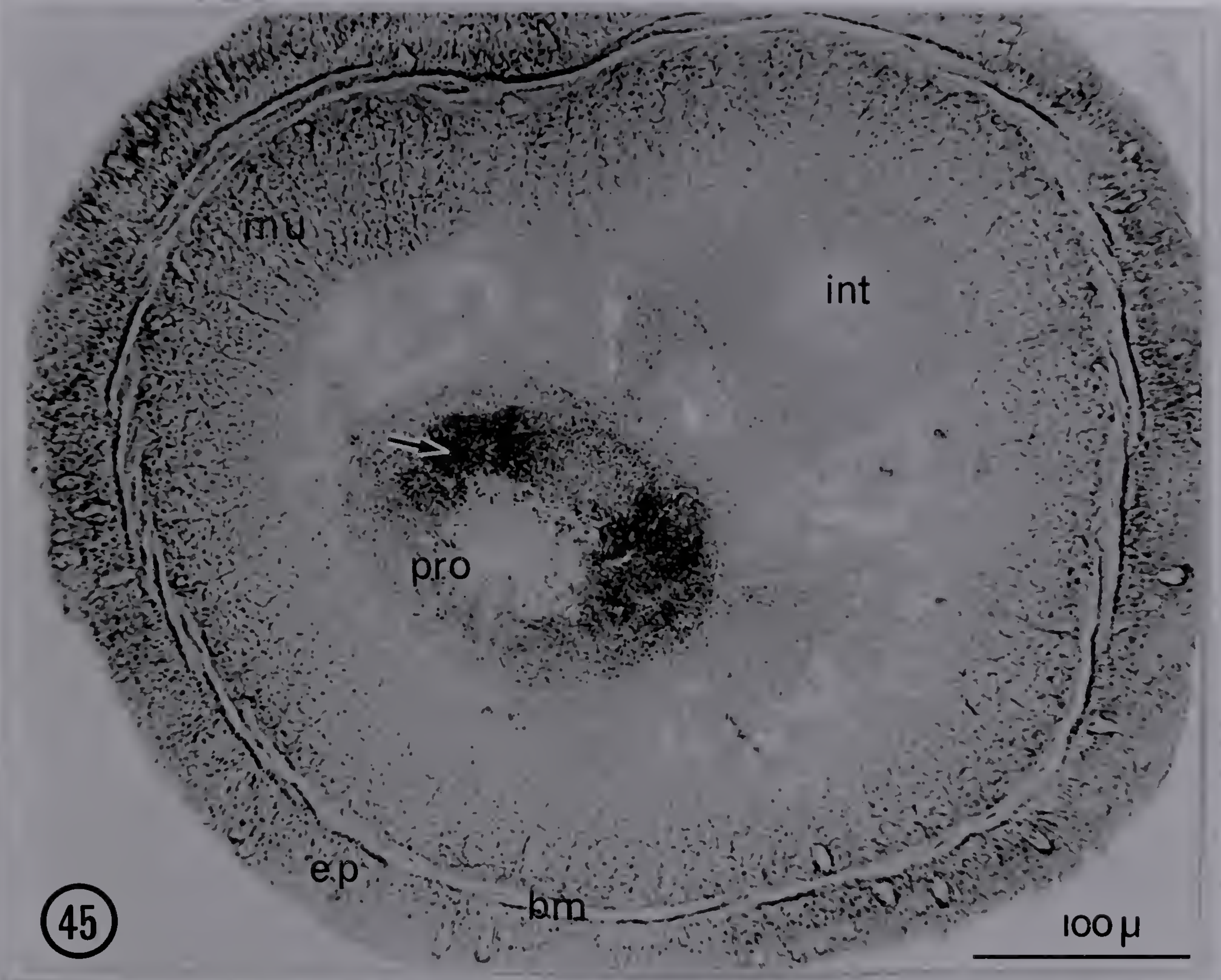
bm, basement membrane. ep, epidermis. int, intestine. mu, muscle. pro, proventriculus.

Fresh frozen section, α-naphthyl phosphate, pH 4. Garnet GBC.

PLATE 46. Transverse section through stomach epithelium. Note infolding of epithelial surface. Lipid globules (arrows) scattered abundantly throughout apical cytoplasm.

bm, basement membrane. lu, lumen of stomach.

Fresh frozen sections, Sudan IV.



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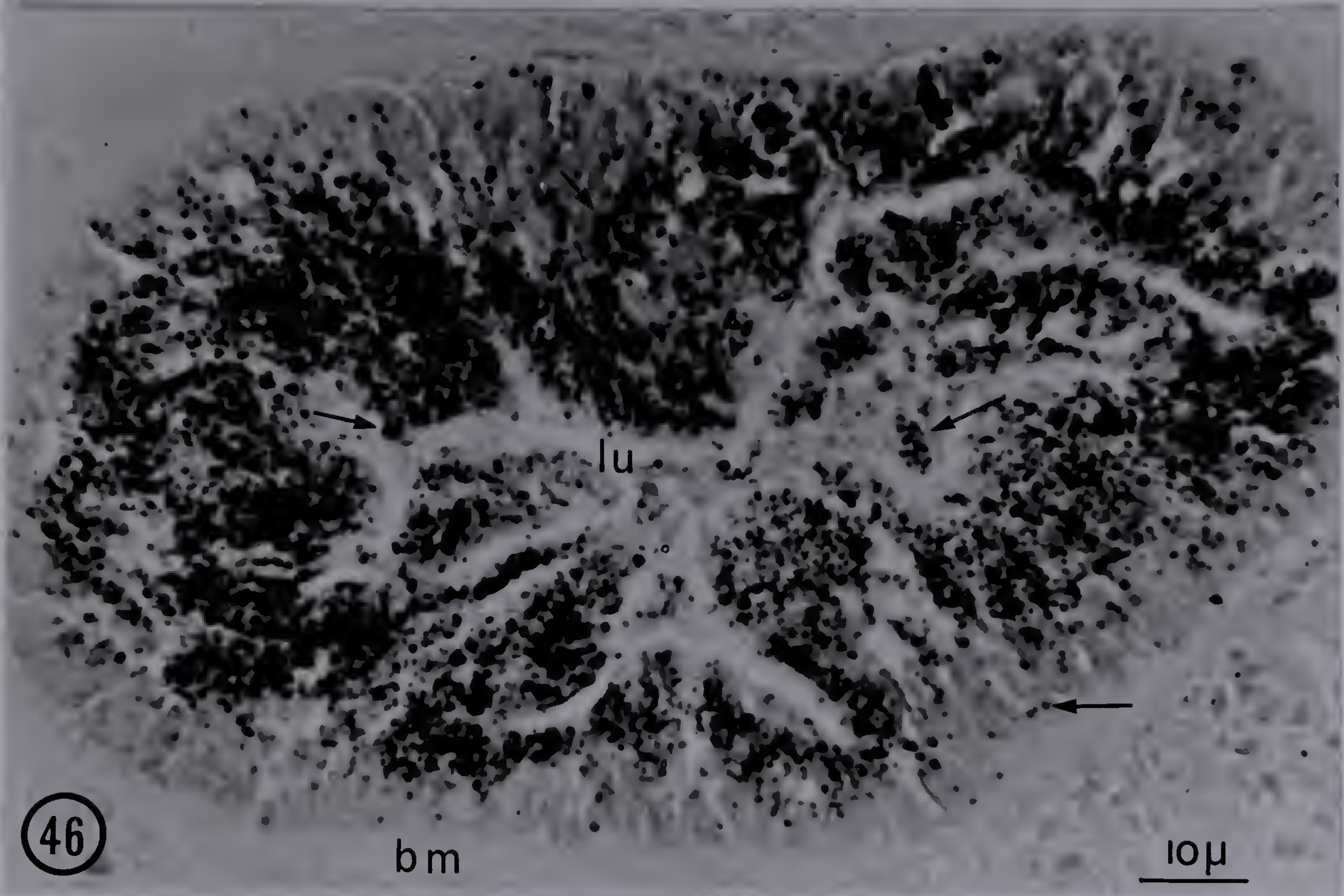

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